UNIVERSIDADE FEDERAL DO PARANÁ

TIAGO SIMÕES MALUCELLI

INTERAÇÃO ENTRE PLANTAS E BEIJA-FLORES EM DIFERENTES ESCALAS ESPACIAIS

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Orientadora: Prof.ª Dr.ª Isabela Galarda Varassin

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RESUMO

Interações entre plantas e polinizadores são essenciais para a maioria das Angiospermas e para um grande número de espécies animais. Dessa forma, o entendimento dos mecanismos que controlam a ocorrência das interações nas comunidades biológicas é imprescindível para a conservação dessa importante função ecossistêmica. Nessa tese, o objetivo geral foi de contribuir para o entendimento de como diferentes fatores determinam o padrão de distribuição das interações planta-polinizador dentro de comunidades e entre comunidades. No Capítulo 1, investigamos como interações beija-flor-planta variam entre localidades na região Neotropical. O *turnover* de interações aumentou rapidamente ao longo de gradientes ecológicos, devido a um rápido *turnover* de plantas e beija-flores. O *turnover* de interações foi melhor predito por diferentes variáveis ecológicas em diferentes domínios biogeográficos situados dentro da região Neotropical, evidenciando não estacionaridade em padrões macroecológicos de *turnover* de interações. A forte associação do *turnover* de interações com gradientes ecológicos reflete um possível efeito da história biogeográfica e de filtros ambientais atuando no passado e no presente, na distribuição de plantas e beija-flores. No Capítulo 2, investigamos a importância relativa do esforço amostral temporal e espacial para a descrição de uma rede beija-flor-planta localizada na Mata Atlântica brasileira. Os resultados mostraram uma maior importância do esforço amostral temporal para a detecção de interações. Apesar da menor importância do esforço amostral espacial, uma reduzida intensidade no esforço espacial é ainda necessária, facilitando a observação de interações em plantas menos na área de estudo. A conectância das redes mostrou uma diminuição enquanto o H_2' foi relativamente estável ao longo do gradiente de esforço amostral espacial e temporal. Nossos resultados reforçam a existência de um forte efeito amostral, majoritariamente relacionado ao esforço temporal, em observações de

interações entre plantas e polinizadores em campo. Além disso, enfatizamos a robustez do índice H_2' para a descrição da especialização da rede. No Capítulo 3, usamos dados de interação entre plantas e beija-flores, provenientes de uma rede de interação local da Mata Atlântica brasileira, para investigar se, em cenários de perturbação no determinantes de interação, a manutenção de determinantes de interação relacionados a morfologia ou fenologia das espécies reduziria a modificação na distribuição das probabilidades de interação na rede. Além disso, buscamos entender como a probabilidade de interação das espécies seriam modificadas quando esses determinantes fossem mantidos sem perturbação. Os resultados mostraram consequências contrastantes. Por um lado, mantendo determinantes morfológicos e fenológicos não perturbados reduz a mudança geral nas probabilidades de interação da rede de interação. No entanto, para espécies que possuem especialização nos atributos morfológicos e fenológicos relacionados à polinização, isso pode resultar em um maior rico de drástica redução nas oportunidades de interação. Isso pode impedir um rápido processo de adaptação em situações de perturbação adversa.

Palavras-chave: Brasil. Esforço amostral. Floresta Atlântica. Gradientes ecológicos. Paraná. Perturbação. Polinização. Redes de interação. *Turnover* de interações.

ABSTRACT

Plant-pollinator interactions are essential for most flowering plants and a great number of animal species. In this way, the elucidation of the mechanisms that control the occurrence of pollination interactions across communities is important for the conservation of this ecosystem function. Currently, we know that many factors act in complex ways to determine which species will interact across ecological communities. In this thesis, our general objective was to contribute to the knowledge on how different factors act to determine the pattern of interactions distribution within and across communities in different spatial scales. In the Chapter 1, we investigated how plant-hummingbird interactions vary across interaction networks in the Neotropical region. Interaction turnover between networks increased sharply along ecological gradients due to rapid plant and hummingbird species turnover. We found different predictors acting as main factors across different biogeographical units, revealing the importance of spatial non-stationarity at macroecological patterns of interaction turnover. The strong association of the interaction turnover with ecological gradients possibly reflects species biogeographical history and the contemporary or past effects of environmental filters. In the Chapter 2, we investigated the relative importance of temporal and spatial sampling effort for the description of a local Brazilian Atlantic rainforest plant-hummingbird interaction network. Our results showed that the temporal sampling effort had a major role on the detection of unique interactions. Although the spatial sampling effort had a minor influence, we showed that small intensities of spatial effort are still important, facilitating the observation of interactions on less common plants. The connectance showed a small decrease while the H_2' was more stable along the spatial and temporal sampling effort gradient. Our results reinforce the existence of strong sampling effects in description of interaction networks, primarily related to the temporal effort when

observing pollination interactions in the field. Furthermore, they emphasize the robustness of the *H2'* specialization index for the description of interaction networks. In the Chapter 3, we used interaction data from a local Atlantic rainforest plant-hummingbird network (the same as in Chapter 2) to understand if, in scenarios where the interaction drivers were perturbed, the maintenance of morphological and phenological interaction drivers unperturbed would reduce the overall modification on the distribution of interaction probabilities. Moreover, we aimed to understand how different species interaction probabilities would change when maintaining these drivers unperturbed. Our results showed contrasting consequences on the robustness from changes on pollination-related traits following perturbations. In one hand, keeping morphological and phenological drivers unperturbed reduced the general modification on interaction probabilities within the network. Nevertheless, for species that are specialized on their morphological and phenological traits, this might result in na enhanced risk of the interaction chance.

Key words: Atlantic rainforest. Brazil. Ecological gradients. Interaction networks. Interaction turnover. Paraná. Perturbation. Pollination. Sampling effort.

SUMÁRIO

1 INTRODUÇÃO GERAL

A ecologia é considerada uma das mais antigas formas de ciência, e suas raízes datam de muito antes da formalização do termo que dá nome a essa área de estudo (WORSTER, 1977). Ao longo da história, uma grande quantidade teorias e modelos conceituais foram desenvolvidos para explicar os padrões de diversidade biológica (PALMER, 1994; VELLEND, 2010). No entanto, a complexidade de sistemas ecológicos, principalmente em nível de comunidade, leva a um grande desafio para o surgimento de leis universais (LAWTON, 1999). Desde a famosa publicação de Lawton (1999) "Are there general laws in ecology?", diversos esforços tem sido feitos para organizar os conceitos ecológicos em teorias mais unificadas (e.g. CHESSON, 2000; VELLEND, 2010).

Nas últimas décadas, as interações entre plantas e polinizadores, foco principal dessa tese, tem sido intensamente estudada através da análise de redes de interação. Muitos mecanismos tem sido propostos para explicar as interações entre as espécies e a variação das mesmas entre comunidades. Sabe-se que processos relacionados ao nicho das espécies são importantes na estruturação das redes de interação. Diversos estudos mostram que características fenotípicas das espécies, como atributos morfológicos e fenológicos, são importantes determinantes da probabilidade de ocorrência das interações e os padrões estruturais em redes ecológicas (JORDANO, 2003; MARUYAMA *et al.*, 2014; REZENDE; JORDANO; BASCOMPTE, 2007; SANTAMARÍA; RODRÍGUEZ-GIRONÉS, 2007; STANG *et al.*, 2009; STANG; KLINKHAMER; VAN DER MEIJDEN, 2006, 2007; VIZENTIN-BUGONI; MARUYAMA; SAZIMA, 2014). A relação entre a probabilidade de interações e atributos das espécies pode ser direta, como por exemplo no caso de barreiras ou encaixes morfológicos entre as espécies (MARUYAMA *et al.*, 2014; SANTAMARÍA;

RODRÍGUEZ-GIRONÉS, 2007; STANG *et al.*, 2009; STANG; KLINKHAMER; VAN DER MEIJDEN, 2006, 2007; VIZENTIN-BUGONI; MARUYAMA; SAZIMA, 2014). No entanto, essa relação pode ser indireta, como é o caso de atributos fenológicos, os quais determinam a distribuição temporal das espécies, influenciando indiretamente a probabilidade de interação (MARUYAMA *et al.*, 2014; VÁZQUEZ, D. P.; CHACOFF; CAGNOLO, 2009; VIZENTIN-BUGONI; MARUYAMA; SAZIMA, 2014). Em outro extremo, muitos estudos tem mostrado que a chance de encontro entre indivíduos de espécies, determinado pelas suas abundâncias relativas, pode determinar fortemente as frequências de interação, e os padrões emergentes das redes ecológicas, revelando a importância de processos ditos neutros (KRISHNA *et al.*, 2008; VÁZQUEZ, D. P.; CHACOFF; CAGNOLO, 2009; VÁZQUEZ, DIEGO P. *et al.*, 2007; VÁZQUEZ, DIEGO P.; AIZEN, 2004).

Os processos neutros e relacionados ao nicho das espécies atuam de forma combinada para a determinação das interações entre plantas e polinizadores, com uma importância variável. Assim, cada rede pode ser contextualizada dentro de um contínuo entre um controle totalmente neutro e um controle totalmente baseado no nicho (CANARD *et al.*, 2012; VIZENTIN-BUGONI *et al.*, 2018). Os motivos pelos quais existe uma variação no balanço entre fatores neutros e de nicho em redes de interação ecológica ainda são debatidos. Recentemente Vizentin-Bugoni*et al.*(2018) propuseram um modelo conceitual para redes de polinização, onde mecanismos neutros seriam mais importantes em comunidades formadas por uma baixa diversidade funcional enquanto mecanismos relacionados ao nicho seriam mais importantes em comunidades com alta diversidade funcional.

Apesar de evidências apontarem que a probabilidade de interação entre espécies são fortemente determinadas pelos atributos e a abundância das mesmas, estes

parâmetros são, por sua vez, influenciados por fatores que atuam em escalas maiores e interagem entre si de maneiras complexas (BARTOMEUS *et al.*, 2016; VÁZQUEZ, D. P. *et al.*, 2009). Dessa forma, a história evolutiva, eventos históricos, a dispersão e o efeito de filtros ambientais, são exemplos de fatores que influenciam a composição e abundância das espécies e de seus atributos, contribuindo para a determinação dos padrões de interação atuais (REZENDE; JORDANO; BASCOMPTE, 2007; TYLIANAKIS; MORRIS, 2017; VITÓRIA; VIZENTIN-BUGONI; DUARTE, 2017). Além disso, perturbações antrópicas que afetam atributos e a distribuição das espécies no espaço e no tempo tem-se mostrado importantes fatores para mudanças nos padrões de interação, podendo levar a efeitos prejudiciais para a persistência das espécies (HEGLAND *et al.*, 2009; MEMMOTT *et al.*, 2007; TYLIANAKIS *et al.*, 2008). Por fim, é amplamente reconhecido que as interações observadas em estudos de campo são amostras a partir de toda a gama de interações que ocorrem em uma determinada comunidade. Assim, efeitos amostrais podem influenciar os padrões de interação observados e levar a interpretações equivocadas (FRÜND; MCCANN; WILLIAMS, 2016; NIELSEN; BASCOMPTE, 2007; VIZENTIN-BUGONI *et al.*, 2016). A elucidação de como esses diferentes mecanismos atuam em conjunto é um importante passo para a conservação dessa importante função ecossistêmica.

Nesse contexto, o objetivo geral dessa tese foi de contribuir para o entendimento de como diferentes fatores atuam e interagem entre si para formar o padrão de distribuição das interações nas comunidades ecológicas. No Capítulo 1 buscamos entender como as interações entre plantas e beija-flores variam no espaço, em escala continental. Nos perguntamos se existe um efeito de variáveis ecológicas no *turnover* de interações, agindo através do *turnover* de espécies ou através do *rewiring* de interações. No Capítulo 2 investigamos a importância relativa do esforço amostral temporal e

espacial para a descrição das interações par-a-par e para os padrões de especialização de uma rede de interação beija-flor planta local. Utilizando a mesma rede de interações simulamos, no Capítulo 3, crescentes perturbações nos determinantes de interações par-a-par para investigar se determinantes de interação relacionados aos atributos das espécies diminuem a desestruturação da rede e se isso poderia causar efeitos negativos para espécies que possuem atributos restritivos.

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CAPÍTULO 1

PLANT-HUMMINGBIRD INTERACTION TURNOVER ACROSS LARGE SCALE ECOLOGICAL GRADIENTS IS MEDIATED BY SPECIES TURNOVER, NOT INTERACTION REWIRING

2 CAPÍTULO 1 - PLANT-HUMMINGBIRD INTERACTION TURNOVER ACROSS LARGE SCALE ECOLOGICAL GRADIENTS IS MEDIATED BY THE SPECIES TURNOVER, NOT INTERACTION REWIRING*

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2.1 ABSTRACT

The drivers of the distribution of species richness have been studied for centuries. Nevertheless, relatively little is known about the distribution of plant-pollinator interactions across the globe. Here, we assessed the relative importance of species turnover and interaction rewiring to plant-hummingbirds interaction beta diversity. Moreover, we investigated the importance of ecological gradients to the turnover of plant-hummingbird interactions. We performed analysis using 71 networks within the Neotropical region and three subsets of these networks, considering smaller extent, named here as biogeographical dominions (Chacoan, Pacific and Parana dominion). We surveyed the relationship between the interaction turnover with ecological gradients using the Generalized Dissimilarity Modeling approach (GDM). In all analysis, interaction turnover was strongly determined by the turnover of species, confirming the expectation of species turnover being more important than rewiring in large scales. Interaction turnover increased sharply along ecological gradients due to rapid plant and hummingbird species turnover. At smaller spatial distances, the turnover of interactions was caused mainly by high turnover of plant species, more than hummingbirds. We found different gradients as main factors across the analysis, revealing the importance of spatial non-stationarity for macroecological patterns of interaction beta diversity. In the Neotropical region, the steep increase on the hummingbird-driven interaction turnover between networks separated by continental distances possibly reflects hummingbird's biogeographical history. Likewise, at the Pacific dominion, higher importance of the geographic distance and elevation gradient might reflect the influence of historical events, including the uplifting of the Andes. In the Parana and Chacoan dominion, the importance

of precipitation and temperature gradients might reflect the contemporary or past effects of environmental filters.

Keywords: Biogeographical regions, ecological gradients, interaction betadiversity, macroecology, pollination.

2.2 INTRODUCTION

Plant-pollinator interactions comprise a key ecosystem process, crucial for the reproduction of most flowering plants and for the subsistence of numerous animal species (Kearns et al. 1998, Ollerton et al. 2011, Rech et al. 2016). Thus, understanding the diversity patterns of plant-pollinator interactions is highly relevant for biodiversity conservation efforts (Harvey et al. 2017). Despite its importance, and contrarily to centuries of research on the causes of species richness patterns (Rahbek et al. 2007), our knowledge on how the distribution of plant-pollinator interactions change across the globe is still on its infancy (Burkle et al. 2016, Rech et al. 2016).

During the last decades, the study of communities of plants and their animal pollinators have attempted to close this knowledge gap by applying network theory, which has led to the discovery of general structural patterns (for reviews see Bascompte and Jordano 2007, Vázquez et al. 2009a). Numerous studies have tried to associate emergent network properties on pollination networks to macroecological gradients, but with contrasting patterns (e.g. Olesen and Jordano 2002, Dalsgaard et al. 2011, Schleuning et al. 2012). Albeit the intense search for patterns in interaction network structure, there is little information on how and why species interactions change along large ecological gradients. The variation of interactions across large ecological gradients might be associated to mechanisms that control the assembly of plants and pollinators in communities, as environmental or dispersal filters (Bartomeus et al. 2016, Tylianakis and Morris 2017). In this way, by studying how the turnover of interaction occurs across these gradients, and considering local interaction drivers, we might improve the ability on predicting how interactions vary on space (Poisot et al. 2015).

Why may an interaction between plant *i* and pollinator *j* occur at one location but not at others? The simpler explanation is that the distributions of the two species is not overlapping.at a given location. In this case, we can say that the interaction *i-j* occur in one location but not in others due to plants or animal pollinator species turnover across space (Novotny 2009, Poisot et al. 2012). Another possibility is the interaction rewiring, where a plant-pollinator pair interact at one location, but not at other, even though these species are present in both locations (Novotny 2009, Poisot et al. 2012). One interesting question, in this sense, is how the balance of the interaction turnover explained by the rewiring or by the species turnover changes on different scales and how this is associated with ecological gradients (Burkle et al. 2016). Recently, researchers started to use beta diversity measures (Anderson et al. 2011) to study patterns of interaction turnover across space and time (Poisot et al. 2012, Carstensen et al. 2014, Simanonok and Burkle 2014, Trøjelsgaard et al. 2015, CaraDonna et al. 2017). Beta diversity measures have proven to be useful on detecting patterns and underlying processes on the distribution of species (e.g. Condit et al. 2002, Tuomisto et al. 2003). Thus, its application on plant-pollinator interaction studies brings a new perspective, which might reveal new insights into how and why interactions changes across space. At small spatial and temporal scales, rewiring has been found an important component for the turnover of interactions (Carstensen et al. 2014, Simanonok and Burkle 2014, CaraDonna et al. 2017). Moreover, the interaction between plants and pollinators seems to change with different intensities over short environmental gradients (Burkle and Alarcón 2011, Simanonok and Burkle 2014), geographical distance (Carstensen et al. 2014) and temporal distance (Simanonok and Burkle 2014, CaraDonna et al. 2017). On the other hand, at large spatial scale, Trøjelsgaard et al. (2015) found a threshold of 450 km beyond which plant-insect interaction turnover between plots is solely caused by

species turnover. Poisot et al. (2017), has found that the turnover of hosts, parasites and their interactions, in a continental extension, is best predicted by the same environmental factors. Nonetheless, the interaction turnover was also driven by variables that do not affect the species turnover, suggesting the direct effect of the environmental turnover on the interactions, leading to interaction rewiring.

Although the recent interest on interaction turnover, we still lack investigations to elucidate how the turnover of interactions acts at macroecological scales (Burkle et al. 2016). By increasing the scale, it is important to evaluate the role of scale dependent responses (Steinbauer et al. 2012, Burkle et al. 2016). As one may expect, at different spatial extents, different diversity patterns might be detected (Nogués-Bravo et al. 2008, Heino et al. 2015). Moreover, one needs to beware of the possibility of detecting different patterns depending on the region used in the analysis (spatial non-stationarity) (Osborne and Suarez-Seoane 2002, Fitzpatrick et al. 2013).

Here we use plant-hummingbird interaction networks in an effort to understand how and why pollination interactions change across large ecological gradients. We intended to answer two questions: (i) what is the relative importance of species turnover and interaction rewiring for the beta diversity of interactions? (ii) is the interaction turnover associated to ecological gradients? We answered these questions by analyzing 71 plant-hummingbird interaction networks within the Neotropical region and 3 subsets of these networks, considering smaller extent, named here biogeographical dominions (Chacoan, Pacific and Parana dominion). We expected the interaction turnover to be always high and mainly determined by the species turnover, since . Moreover, we expected interaction turnover to increase fast with the spatial and environmental distance between locations and that this increase would be related to the effect of the environmental and spatial distances on the turnover of plants and

pollinators across the networks (Trøjelsgaard et al. 2015). An alternative situation would be one where the interaction turnover is associated to the environmental and spatial distance between locations, but this is not related to a turnover of plants and hummingbirds, what would suggest that the effect of environmental and spatial dissimilarities acts directly on the interactions, leading to interaction rewiring (Poisot et al. 2017).

We anticipated that the predominant ecological factors enrolled on the species mediated interaction turnover would be different between the biogeographical units (Osborne and Suarez-Seoane 2002, Nogués-Bravo et al. 2008, Fitzpatrick et al. 2013, Heino et al. 2015). At the Neotropical region, we predicted an enhanced importance of the geographic distance, since we would be including networks that are spatially very distant, showing a possible imprint of different historical factors (Ricklefs 2004). Within the biogeographical dominions, we predicted that contemporary environmental factors would be more important to describe the interaction turnover, but that these would vary in importance depending on the dominion analyzed. We expected plants and hummingbirds to respond differently to these environmental gradients (Arroyo et al. 1982). Specifically, we hypothesized that the interaction turnover driven by hummingbird's turnover would be mainly affected by elevation and temperature gradients, since these variables seem to be related to hummingbird's biogeographical history and current diversity (Bartholomew et al. 1957, Lasiewski and Lasiewski 1967, Calder 1971, Rahbek and Graves 2000, Chaves and Smith 2011, Projecto-Garcia et al. 2013, McGuire et al. 2014, Weinstein et al. 2014, Benham et al. 2015, Powers et al. 2017). On the other hand, we hypothesized that the interaction turnover driven by plants turnover would be mainly affected by the precipitation gradient, as it is an important

driver of plants turnover (Benzing 1998, Oliveira-Filho and Fontes 2000, Krishnadas et al. 2016).

2.3 MATERIAL AND METHODS

Data set

We used a database comprising 71 plant-hummingbird interaction networks from several locations within the Neotropical region (Dalsgaard et al. 2011, Martín González et al. 2015; Fig. 1; Table S1). These interaction networks are organized in interaction matrices with plant species in lines and hummingbird species in columns. Each cell of the interaction matrices has information on the frequency of interactions observed between each plant and each hummingbird species.

To control for the interaction turnover due to differential sampling completeness between pairs of networks, we accessed the sampling completeness of each interaction network using a method proposed by Chacoff et al. (2012) and adapted by Devoto et al. (2012). This method consists in calculating the proportion of the observed interaction richness from the estimated interaction richness. To calculate the estimated richness, we used the Chao 1 estimator in the estimateR function at the vegan package (Oksanen et al. 2017). In this case, instead of providing information on the number of individuals of each species, we provided the amount of interaction of each pair of species. We performed all these procedures in the R software (R Core Team 2016).

For each study location, we extracted the elevation using the GMTED2010 layer at a resolution of approximately 1km (https://lta.cr.usgs.gov/GMTED2010). We used this layer to calculate the mean elevation for a buffer of 2km around each location. Additionally, for each location, within a buffer of 10 km, we extracted environmental

information on mean annual temperature, temperature seasonality, mean annual precipitation and precipitation seasonality from the Worldclim database ver.2 (Fick and Hijmans 2017). We used a coarser resolution than elevation for these variables, because the WorldClim interpolation showed substantial errors for high elevations to generate finer resolutions estimates of precipitation and temperature (Fick and Hijmans 2017). We conducted a dimensionality reduction by PCA, merging the annual mean to the annual seasonality, separately for temperature and precipitation variables. High score values at the PCA axis of temperature and at the PCA axis of precipitation describe, respectively, high temperature (low temperature seasonality) and high precipitation (low precipitation seasonality). This strategy permits interpreting the simultaneous effect of the mean and the variation of temperature and precipitation (Krishnadas et al. 2016), while reducing the number of predictors in the models. Throughout the manuscript, we refer to the temperature PCA axis as a variable called "temperature" and the precipitation PCA axis as a variable called "precipitation". We calculated the Euclidean distance for the above variables between plots using the Vegan package (Oksanen et al. 2017) and the geographical distance between plots using the Geosphere package, (Hijmans 2017) to access if they were correlated. We used distances to access variables correlation because this is the form they will be used at the modelling procedures (see below). If two predictors had a correlation higher than 0.7 we excluded one of them.

Beta diversity of interactions

We measured the interaction turnover between a pair of interaction networks using the Jaccard index:

$$\beta cc = \frac{(b+c)}{(a+b+c)} \quad (1)$$

where **b** is the number of unique interactions present only in the first network, **c** is the number of unique interactions present only in the second network and **a** is the number of unique interactions shared by both networks (Novotny 2009, Simanonok and Burkle 2014). The interaction turnover based on the Jaccard index can be partitioned in four components (Novotny 2009, Simanonok and Burkle 2014):

$$\beta cc = \beta p + \beta h + \beta ph + \beta 0 = \frac{(bp+cp)}{(a+b+c)} + \frac{(bh+ch)}{(a+b+c)} + \frac{(bph+cph)}{(a+b+c)} + \frac{(b0+c0)}{(a+b+c)} \quad (2)$$

The βp component is the proportion of the interactions that are lost from one network to another because the plants involved at these interactions are lost. The βh component is the proportion of the interactions that are lost from one network to another because the hummingbirds involved at these interactions are lost. The βph component is the proportion of the interactions that are lost from one network to another because the hummingbirds and plants involved at these interactions are lost simultaneously. The β0 component is the proportion of the interactions that are lost from one network to another because shared species interact in just one of the networks (i.e. due to interaction rewiring). The sum of the components βp, βh and βph originates the βst component, which represents the proportion of interactions that are lost from one network to another because of the species turnover between them. To calculate the beta diversity of interactions and its components we used codes provided by Simanonok and Burkle (2014) on the R software (R Core Team 2016).We created two additional components to describe the entire proportion of interactions that change due to plants (βph + βp = βpph; plant-driven interaction turnover) or due to hummingbirds (βph + βh = βhhp; hummingbird-driven interaction turnover) turnover. We stress that, in this framework, the loss of an interaction because a species is lost does not necessarily means the complete absence of the species in one locality, but that it was not observed interacting.

Statistical analysis

We performed the statistical analysis using the 71 plant-hummingbird quantitative interaction networks for the whole Neotropical region and three subsets of them within different biogeographical dominions (Fig. 1; Table S1; Morrone 2014a). This biogeographical classification was based on different taxa of terrestrial plants and animals (Morrone 2014b). We used a biogeographical regionalization approach as it has the potential to reveal particularities on how the biodiversity is structured across different biogeographical regions (Hawkins et al. 2003). We chose three dominions that had at least 12 interaction networks within its limits: Parana dominion (n=16); Chacoan dominion (n=12); Pacific dominion (n=17; Fig. 1), using a shapefile containing the biogeographical regions limits (Löwenberg-Neto 2014). For each biogeographical unit (Neotropical region and the three biogeographical dominions), we partitioned the interaction beta diversity in the component that represents the interaction turnover related to the species turnover (βst) and in the component that represents the interaction turnover related to the interaction rewiring (β0). Thus, we could investigate the relative contribution of each of these components to the beta diversity of interactions.

To understand the relationship of the interaction beta diversity, with the predictor variables, we used the Generalized Dissimilarity Modelling (GDM) framework (Ferrier et al. 2007). This method is widely applied to model the beta diversity of species, especially in greater ecological gradients (Fitzpatrick et al. 2013, Loiseau et al. 2017). GDM accounts for non-linear patterns of biological dissimilarity (Faith et al. 1987) and non-constant rates of biological turnover, along ecological gradients (Simmons and Cowling 1996). The non-linear patterns of biological dissimilarity is resolved by applying General Linear Modelling (Millar et al. 2011). We used the link function recommended by Ferrier et al. (2007):

$$d_{ij} = 1 - e^{(b+E_{ij})} \quad (3)$$

where d_{ij} is the predicted biological dissimilarity between two localities, E_{ij} is the ecological distance between two localities (sum of the absolute differences of the transformed predictors) and b is the intercept of the model. To address non-constant rates of turnover along ecological gradients, the solution applied by GDM is to promote non-linear transformations on the predictor variables to maximize the fit between the biological turnover and the predictor variables dissimilarities. We realized three GDM analyses for each biogeographical unit, using as response variables the beta diversity of interactions (βcc), the plant-driven interaction turnover (βpph) and the hummingbird-driven interaction turnover (βhhp). We used as predictor variables the distance between all combinations of networks in terms of the PCA axis of temperature, the PCA axis of precipitation, the elevation, the geographic position and the sampling completeness. For the Chacoan dominion we excluded the elevation, as elevation differences were strongly correlated to temperature differences between plots (r=0.74). Additionally, we excluded the geographic distance that was correlated with precipitation dissimilarities (r=0.83). We calculated the explained deviance for each model by dividing the difference between the null deviance and residual deviance by the null deviance (Zuur et al. 2009). The significance of each model was accessed by comparing the explained deviance of each model with the explained deviance of the same model, but using data generated by 1000 rounds of a null model (described below). To test the importance of each predictor for the full model fit, we accessed the loss of fitness magnitude (increase of deviance) when dropping each variable from the full model (Ferrier et al. 2007, Loiseau et al. 2017). The same procedure was repeated for each round of the null model. We considered significant variables that showed an importance higher than 95% of the

importance obtained by the null model. We ran these analyses using the GDM package (Manion et al. 2017) on the R software (R Core Team 2017).

Null model

Patterns of species beta diversity arise by processes that cause the lost or gain of species, leading to differences on species richness, and by processes that cause the replacement of species (Baselga 2010). To verify if the interaction beta diversity could be explained solely by species and interaction richness differences between localities, we created a null model based on Chase et al. (2011), including a step to wire interactions between plants and hummingbirds (Trøjelsgaard et al. 2015). First, we assembled plants and hummingbirds in each locality with a probability proportional to each species occupancy within the biogeographical region being analysed, constraining each locality network richness of plants and hummingbirds (Chase et al. 2011). We defined occupancy as the number of networks each species occurred (Chase et al. 2011). The second step was to make species interact within localities with a probability proportional to its occupancy values (Trøjelsgaard et al. 2015). With this null model, our intention was to control for the interaction turnover that is caused only by differences of species and interaction richness. Thus, if the observed interaction turnover is better described by the ecological distance than the null interaction turnover, we interpreted that the predictors influenced the interaction turnover not only by differences of richness, but by a strong effect of the replacement of species and interactions as well.

2.4 RESULTS

In general, the interaction turnover was tightly mediated by the turnover of species between networks (Fig. 2), increasing fast across the ecological gradient (Fig. 3a, 4a, 5a, 6a). In all biogeographic units, even environmentally and geographically close networks showed a high plant driven interaction turnover, what is proven by the high intercepts when using the βpph component as response on the models (Fig. 3b, 4b, 5b, 6b). Contrary to that, the models predicting the hummingbird driven interaction turnover showed much lower intercepts (Fig. 3c, 4c, 5c, 6c). This difference could arise because of a higher variation on the plant richness between networks compared to the hummingbird's richness (Fig. S1). Nonetheless, the same intercept difference for the null models, that should reflect the difference? expected by richness differences, are lower (Table S2), showing that the higher plant-driven interaction turnover intercepts reflects a fast interaction turnover driven by plants replacement between networks.

The relevance of ecological predictors changed across the biogeographical units. In the Neotropical region, the geographic distance was the best descriptor for the interaction turnover, followed by the plant-driven interaction turnover and the hummingbird-driven interaction turnover (Fig. 3, Table 1). High rates of interaction turnover occurred at the beginning and at the end of the geographic distance gradient for the interaction turnover and for the plant-driven interaction turnover (Fig. 3 a, b). The interaction turnover rate due to hummingbird's turnover showed a sharp increase after a threshold of approximately 4000 km (Fig. 3c). In the Parana dominion, both precipitation and temperature were important to interaction turnover (Fig. 4a, Table 1), with precipitation as the best predictor of plant-driven interaction turnover (Fig. 4b, Table 1) and temperature followed by precipitation for hummingbird-driven interaction turnover (Fig. 4c, Table 1). In the Chacoan dominion, temperature and precipitation explained interaction and plant-driven interaction turnover (Fig. 5a, b, Table 1). The

hummingbird-driven interaction turnover was best explained by the temperature, but the precipitation was relatively influent as well, with both showing similar trends on the rate of turnover (Fig 5c, Table 1). Finally, for the Pacific dominion, we found a strong role of the geographic and elevation distance on interaction turnover as well as plant- and hummingbird-driven interaction turnover (Figure 6a-c, Table 1). Although both variables were important, the hummingbird-driven interaction turnover better related to the geographic distance, while the plant-driven interaction turnover to elevation differences (Figure 6a-c, Table 1).

2.5 DISCUSSION

Confirming our expectations, we found that at large spatial extent, turnover of interactions between plants and hummingbirds was determined mainly by the turnover of interacting species. The fact that within all biogeographical units the interaction turnover, and the plant and hummingbird-driven interaction turnover, were successfully predicted by the same ecological variables, shows that these variables contribute largely to the turnover of interactions by acting on the turnover of species and not by causing interaction rewiring (Poisot et al. 2017).

Notably, plant-driven interaction turnover was more important than hummingbird-driven interaction turnover in smaller ecological distances. The fact that the turnover of interactions between networks that are geographically and environmentally close is mainly caused by plants turnover may indicate that, in general, hummingbirds have larger ranges and are less patchy distributed than plants. Moreover, it shows an ability of these birds to interact with plant species from different

communities. Most plant-hummingbird interactions are known to be primarily driven by morphological barriers and phenological coupling (Vizentin-Bugoni 2017). Thus, the ability of hummingbirds to persist in communities with different plants composition would imply the need of a functional redundancy on plants traits related to pollination across communities (Benadi et al. 2014).

We observed that, even between geographically and environmentally similar networks, there was a high plant driven interaction turnover. This point towards the existence of non-included environmental factors that drive the turnover of plants between these networks, leading to high rates of interaction turnover. Moreover, competition or facilitation between plant species could be responsible for this high turnover without the need of high environmental differences (Pellissier et al. 2010, Gutiérrez et al. 2014, Darwell et al. 2017). Although, our results reveal plants and hummingbirds responding in different ways to increasing ecological distance (Arroyo et al. 1982, Trøjelsgaard et al. 2015), this does not exclude the possibility that a proportion of the interaction turnover is actually due to a mutualistic resource tracking of one group to the other (Cotton 2007).

We found that the most important ecological variables varied between biogeographical units, evidencing spatial non-stationarity on the association of these variables to the interaction turnover across the continent (Osborne and Suarez-Seoane 2002). In the Neotropical region, the steep increase on the hummingbird-driven interaction turnover between networks separated by continental distances possibly reflects hummingbird's biogeographical history. Hummingbirds originated at South American humid lowlands, invading and diversifying in the Andes, and more recently in the Caribbean islands and in the North American continent (McGuire et al. 2014). This diversification was likely mediated by an availability of new environments and by

a diffuse coevolution with plants (McGuire et al. 2014). Given that these diversification areas are well represented in our data set we can interpret the high hummingbird-driven interaction turnover as being indirectly associated to the biogeographical history of the hummingbirds. The plant-driven interaction turnover did not show the same sharp increase, probably because plants that are visited by hummingbirds are distributed in several families with different evolutionary histories (Cronk and Ojeda 2008). Nevertheless, we still found a high turnover of interactions due to plants turnover at continental distances, showing a possible footprint of different lineages history. Indeed, Givnish et al. (2014) showed that species of Bromeliaceae, an important resource for hummingbirds (Buzato et al. 2000, Varassin and Sazima 2000, 2012), have great centers of diversification at the North Andes and at the coastal mountains of Brazil. The fact that even smaller geographic distances caused an effect on the interaction turnover due to plants turnover, could be reflecting a current or past process of environmental filtering (Keddy 1992) caused by spatially structured environmental variables (Nekola and White 1999).

A historical imprint on the interaction turnover seems to occur at the Pacific Dominion as well. The great role of geographic distance and elevation gradients on the interaction turnover could be a reflex of past events on the composition of hummingbirds and their nectar sources. Indeed, there are several evidences of past biogeographical events structuring the biological diversity in this region. The most notable are the uplifting of the north region of the Andes, dynamic past climatic variations during the ice age and the connection of North and South America by the Panamá isthmus (Gentry 1982, Hooghiemstra and Van der Hammen 2004, Ribas et al. 2007, Hoorn et al. 2010). This is supported by studies showing that the dispersion and diversification of groups of hummingbirds, and plants that are common resources for

them, are related to biogeographical events in this region (Chaves and Smith 2011, Givnish et al. 2011, 2014, Benham et al. 2015). Moreover, the high elevation difference is also known to be associated to a high turnover of hummingbirds at the north region of the Andes (Weinstein et al. 2014). Noteworthily, hummingbirds adapted to high elevations developed specific traits such as hemoglobin changes to cope with the reduced quantity of oxygen (Projecto-Garcia et al. 2013). Here we provide evidence that a high turnover of hummingbirds in this region reflects on the interactions performed by them as well. To what extent this possible effect of an active biogeographical history on the interaction turnover is also related to a process of diffuse coevolution between plants and hummingbirds (Givnish et al. 2014, McGuire et al. 2014) is unknown, but it has certainly an important contribution.

Climatic differences of temperature and precipitation had higher influence on the interaction turnover in the Parana and Chacoan dominion. At the first, the interaction turnover related to plant turnover is probably related to a change on precipitation regimes from humid coastal rainforests to inland seasonal forests, which is a main driver of the floristic composition at this dominion (Oliveira-Filho and Fontes 2000). It is not a surprise that plants turnover across the networks is closely associated to precipitation variations, since a variety of species are known to respond to it (Krishnadas et al. 2016), including epiphytes (Benzing 1998), a common resource for hummingbirds (Buzato et al. 2000). As hummingbird's driven interaction turnover was also predicted by precipitation, one hypothesis is that hummingbirds could have tracked the change of its plant resources on the precipitation gradient (Cotton 2007). Nonetheless, the interaction turnover due to the turnover of hummingbirds is likewise predicted by the temperature gradient, what could reflect species constraints related to their physiological limits on high and low temperatures (Bartholomew et al. 1957,

Lasiewski and Lasiewski 1967, Calder 1971, Powers et al. 2017). At the Chacoan dominion, the great role of precipitation is probably associated to changes of rainfall regimes from North to South. This is supported by the high correlation between precipitation and geographic distance, since the networks within this dominion have a wide latitudinal distribution. At the northern limit of this dominion, we find networks located at the Caatinga, a biome with low rainfall and substantial seasonality (Nimer 1989). At its central part, we find networks at locations of seasonal precipitation regime but not as extreme periods of drought (Nimer 1989). At its southern portion, we find a network at the Pampa region, that show no marked dry season (Nimer 1989). These three portions has been considered as separate biogeographical provinces (Morrone 2000), supported by different biogeographical hypothesis (Porzecanski and Cracraft 2005, Werneck 2011). Thus, we can interpret our results as being at least partly because of an historical influence of precipitation on the distribution of plants and hummingbirds. The fact that the temperature is highly correlated with elevation shows we might be also capturing a turnover of plants and hummingbirds between geographically close networks but under different temperatures regimes due to elevation differences. Nevertheless, this dominion has a great spatial extent when compared to the others, but a low number of networks. Thus, these conclusions should be taken with caution.

2.6 STUDY LIMITATIONS

It is important to note that our conclusions are constrained by some limitations. One important limitation is that, besides we identified the mutualistic species turnover

as a link between the interaction turnover and ecological dissimilarity, we did not used true species turnover data (species presence or absence surveys). Instead, we used observation of interactions as a proxy for species presence or absence. Although it might not be the best approach, the number of interactions have been used as a proxy of plants and pollinators abundance in many studies (Vázquez et al. 2009b, Maruyama et al. 2014). Moreover, since we did not find strong roles of sampling completeness on the interaction turnover, we are confident that the eventual not sampled species would not change our results. Nevertheless, our results need to be interpreted with caution, since sampling effort have considerable effects in plant-hummingbird networks (Vizentin-Bugoni et al. 2016). Another limitation resides on the fact that networks geographic distribution left some wide geographical gaps. By describing the geographical space more thoroughly, we could have provided conclusions that are more reliable. Unfortunately recording plant-pollinator interactions demand high efforts (Hegland et al. 2010) and we are still far from having extensive data bases. As pointed out by Poisot et al. (2012), network ecologists should discuss more frequently ways to improve these global data sets.

2.7 CONCLUSION

The use of the beta diversity concept to study the variation of interactions in space and time has been gaining increasing attention from ecologists. Nevertheless, several important questions are still unanswered (Burkle et al. 2016). By exploring the interaction beta diversity in a continental scale, we contributed to the understanding of some of these knowledge gaps. Our results confirmed the expectation that the species

turnover is more important than the rewiring in large scales (Burkle et al. 2016). Moreover, we showed an enhanced role of environmental variables and of possible historical events on the distribution of plant-pollinator interactions. Nonetheless, this was specific for each biogeographic region. We recommend future studies to focus on measuring the extent of sampling effects and the influence of data geographical incompleteness at the beta diversity of interactions.

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2.10 FIGURES

Figure 1. Geographical location of the 71 plant-hummingbird interaction networks within the Neotropical region. The three colored areas represent different biogeographic dominions based on Morrone (2014a,b).

Figure 2. Boxplots showing the beta diversity of interactions (βcc) and its species turnover (β_{st}) and rewiring (β_0) components for the (a) Neotropical region, (b) Parana dominion, (c) Chacoan dominion and (d) Pacific dominion

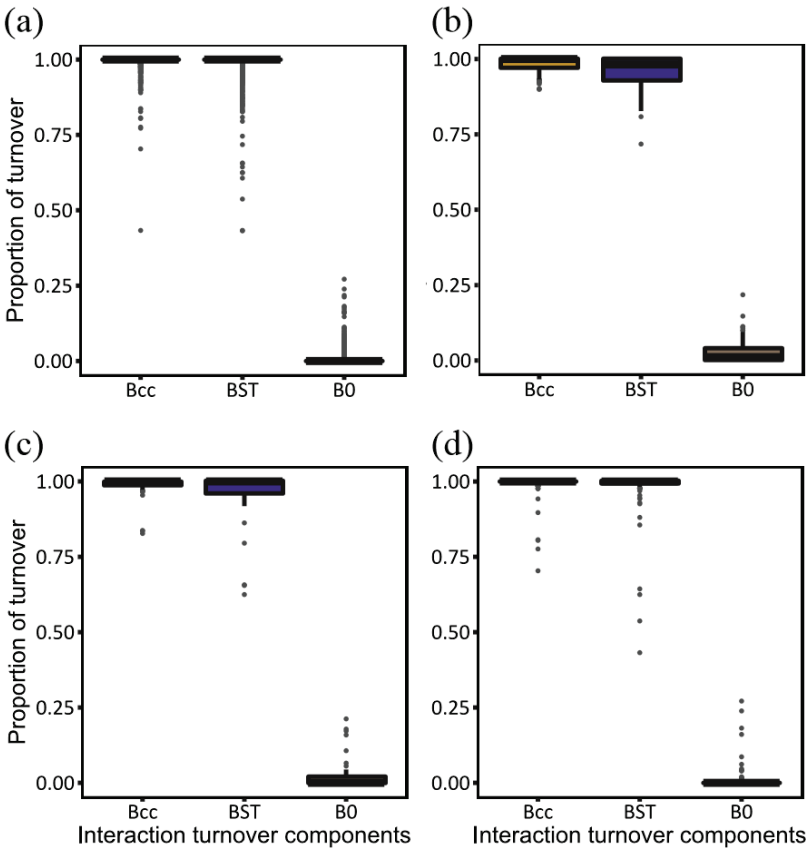


Figure 3. Relationship of the interaction turnover (a), plant driven-interaction turnover (b) and hummingbird-driven interaction turnover (c), with the ecological distance at the Neotropical region. The plots on the left show the full model predicted relationship. *ExpDev* is the explained deviance and p is the significance of the full model. The interaction beta diversity (interaction dissimilarity) is reported on the y-axis and the ecological distance is reported on the x-axis. The two plots on the right show the transformation function only for the two predictors that had the highest importance for the model. The x-axis contains each variable raw value (geographic distance values reported are the raw values in km, divided by 100). The y-axis contains each variable transformed values. The shape of each transformation function represents the rate of turnover related to each variable on different parts of the gradient. *I* is the importance of each variable for the model and *p* is its significance.

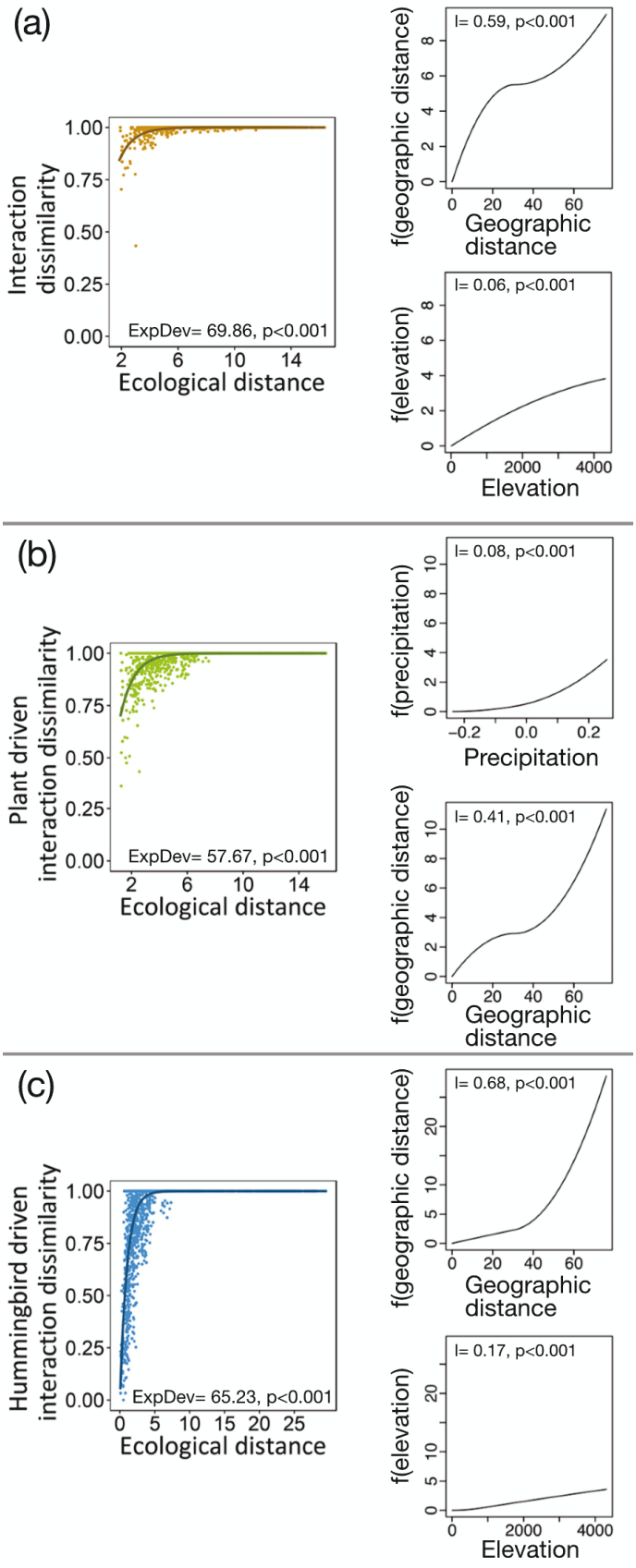

(a)
Interaction dissimilarity
1.00
0.75
0.50
0.25
0.00
ExpDev= 69.86, p<0.001
2
6
10
14
Ecological distance
f(geographic distance)
I= 0.59, p<0.001
0
2
4
6
8
0
20
40
60
Geographic distance
f(elevation)
I= 0.06, p<0.001
0
2
4
6
8
0
2000
4000
Elevation
(b)
Plant driven interaction dissimilarity
1.00
0.75
0.50
0.25
0.00
ExpDev= 57.67, p<0.001
2
6
10
14
Ecological distance
f(precipitation)
I= 0.08, p<0.001
0
2
4
6
8
10
−0.2
0.0
0.2
Precipitation
f(geographic distance)
I= 0.41, p<0.001
0
2
4
6
8
10
0
20
40
60
Geographic distance
(c)
Hummingbird driven interaction dissimilarity
1.00
0.75
0.50
0.25
0.00
ExpDev= 65.23, p<0.001
0
5
10
15
20
25
Ecological distance
f(geographic distance)
I= 0.68, p<0.001
0
5
10
20
0
20
40
60
Geographic distance
f(elevation)
I= 0.17, p<0.001
0
5
10
20
0
2000
4000
Elevation

Figure 4. Relationship of the interaction turnover (a), plant-driven interaction turnover (b) and hummingbird-driven interaction turnover (c), with the ecological distance at the Parana dominion The plots on the left show the full model predicted relationship. *ExpDev* is the explained deviance and p is the significance of the full model. The interaction beta diversity (interaction dissimilarity) is reported on the y-axis and the ecological distance is reported on the x-axis. The two plots on the right show the transformation function only for the two predictors that had the highest importance for the model. The x-axis contains each variable raw value. The y-axis contains each variable transformed values. The shape of each transformation function represents the rate of turnover related to each variable on different parts of the gradient. *I* is the importance of each variable for the model and *p* is its significance.

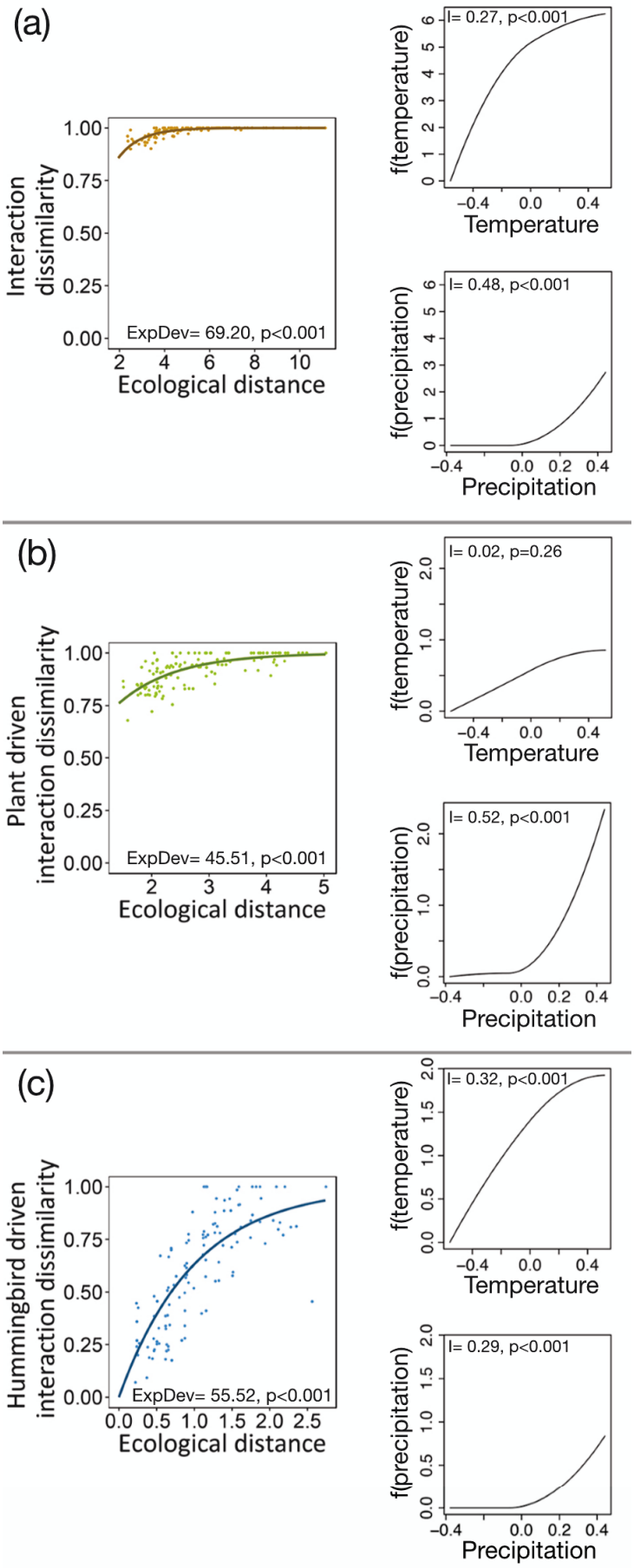

(a)
Interaction dissimilarity
1.00
0.75
0.50
0.25
0.00
ExpDev= 69.20, p<0.001
2 4 6 8 10
Ecological distance
I= 0.27, p<0.001
f(temperature)
0 1 2 3 4 5 6
−0.4 0.0 0.4
Temperature
I= 0.48, p<0.001
f(precipitation)
0 1 2 3 4 5 6
−0.4 0.0 0.2 0.4
Precipitation
(b)
Plant driven interaction dissimilarity
1.00
0.75
0.50
0.25
0.00
ExpDev= 45.51, p<0.001
2 3 4 5
Ecological distance
I= 0.02, p=0.26
f(temperature)
0.0 1.0 2.0
−0.4 0.0 0.4
Temperature
I= 0.52, p<0.001
f(precipitation)
0.0 1.0 2.0
−0.4 0.0 0.2 0.4
Precipitation
(c)
Hummingbird driven interaction dissimilarity
1.00
0.75
0.50
0.25
0.00
ExpDev= 55.52, p<0.001
0.0 0.5 1.0 1.5 2.0 2.5
Ecological distance
I= 0.32, p<0.001
f(temperature)
0.0 0.5 1.0 1.5 2.0
−0.4 0.0 0.4
Temperature
I= 0.29, p<0.001
f(precipitation)
0.0 0.5 1.0 1.5 2.0
−0.4 0.0 0.2 0.4
Precipitation

Figure 5. Relationship of the interaction turnover (a), plant driven interaction turnover (b) and hummingbird driven interaction turnover (c), with the ecological distance at the Chacoan dominion. The plots on the left show the full model predicted relationship. *ExpDev* is the explained deviance and p is the significance of the full model. The interaction beta diversity (interaction dissimilarity) is reported on the y-axis and the ecological distance is reported on the x-axis. The two plots on the right show the transformation function only for the two predictors that had the highest importance for the model. The x-axis contains each variable raw value. The y-axis contains each variable transformed values. The shape of each transformation function represents the rate of turnover related to each variable on different parts of the gradient. *I* is the importance of each variable for the model and *p* is its significance.

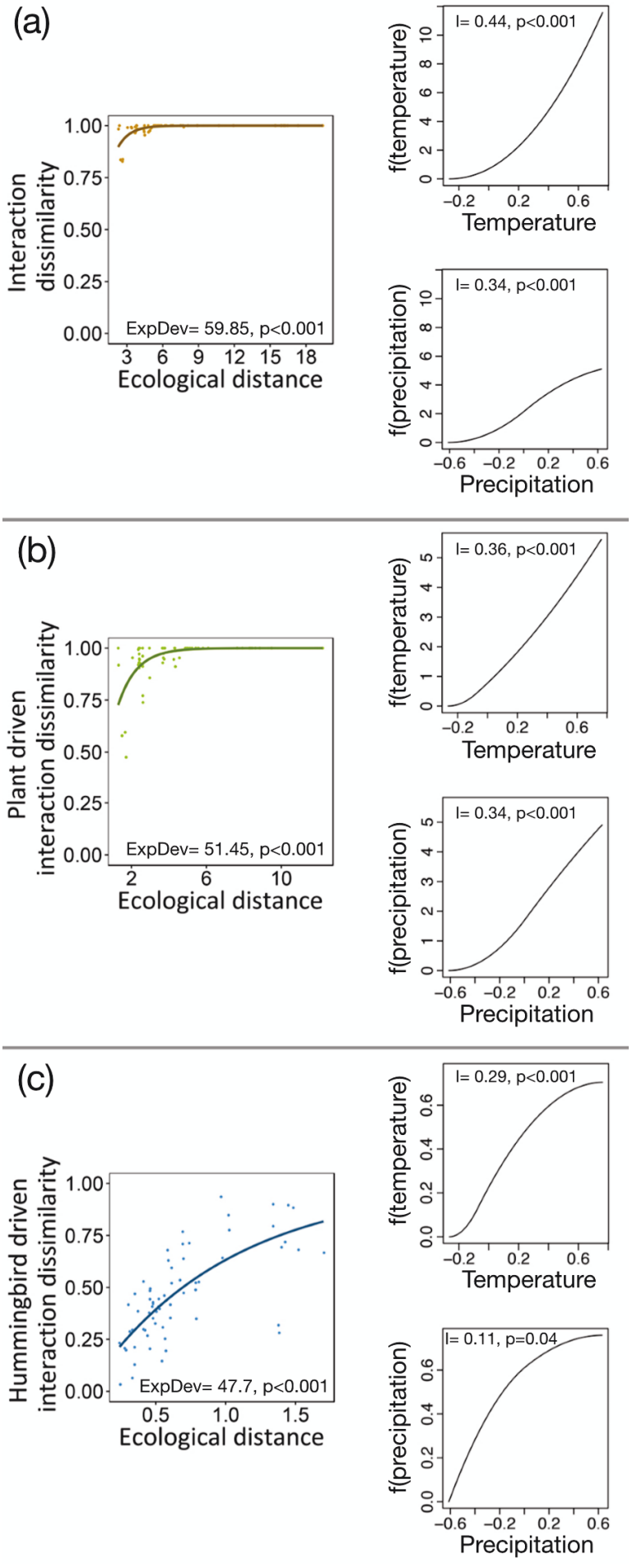
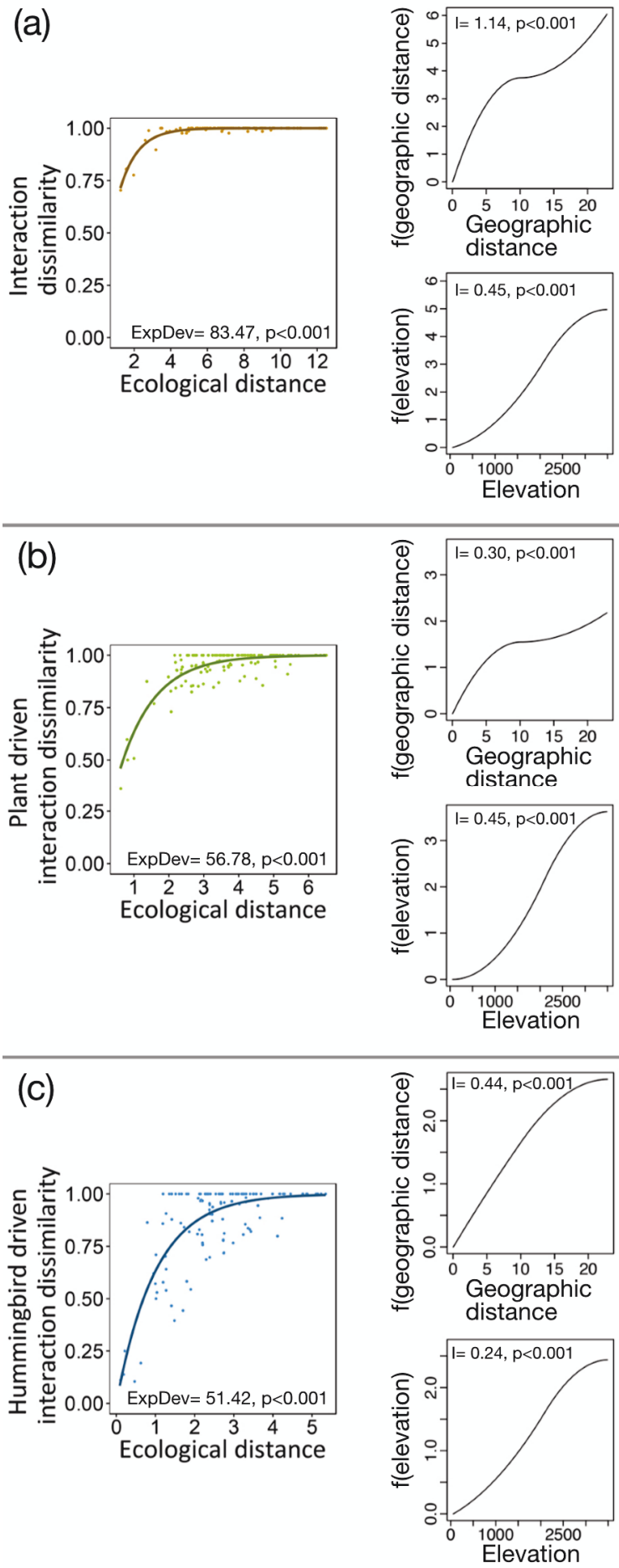

(a)
Interaction dissimilarity
1.00
0.75
0.50
0.25
0.00
ExpDev= 59.85, p<0.001
3 6 9 12 15 18
Ecological distance
f(temperature)
l= 0.44, p<0.001
0 2 4 6 8 10
−0.2 0.2 0.6
Temperature
f(precipitation)
l= 0.34, p<0.001
0 2 4 6 8 10
−0.6 −0.2 0.2 0.6
Precipitation
(b)
Plant driven interaction dissimilarity
1.00
0.75
0.50
0.25
0.00
ExpDev= 51.45, p<0.001
2 6 10
Ecological distance
f(temperature)
l= 0.36, p<0.001
0 1 2 3 4 5
−0.2 0.2 0.6
Temperature
f(precipitation)
l= 0.34, p<0.001
0 1 2 3 4 5
−0.6 −0.2 0.2 0.6
Precipitation
(c)
Hummingbird driven interaction dissimilarity
1.00
0.75
0.50
0.25
0.00
ExpDev= 47.7, p<0.001
0.5 1.0 1.5
Ecological distance
f(temperature)
l= 0.29, p<0.001
0.0 0.2 0.4 0.6
−0.2 0.2 0.6
Temperature
f(precipitation)
l= 0.11, p=0.04
0.0 0.2 0.4 0.6
−0.6 −0.2 0.2 0.6
Precipitation

Figure 6. Relationship of the interaction turnover (a), plant driven interaction turnover (b) and hummingbird driven interaction turnover (c), with the ecological distance at the Pacific dominion. The plots on the left show the full model predicted relationship. *ExpDev* is the explained deviance and p is the significance of the full model. The interaction beta diversity (interaction dissimilarity) is reported on the y-axis and the ecological distance is reported on the x-axis. The two plots on the right show the transformation function only for the two predictors that had the highest importance for the model. The x-axis contains each variable raw value (geographic distance values reported are the raw values in km divided by 100). The y-axis contains each variable transformed values. The shape of each transformation function represents the rate of turnover related to each variable on different parts of the gradient. *I* is the importance of each variable for the model and *p* is its significance.


(a)
Interaction dissimilarity
1.00
0.75
0.50
0.25
0.00
ExpDev= 83.47, p<0.001
2 4 6 8 10 12
Ecological distance
f(geographic distance)
l= 1.14, p<0.001
0 1 2 3 4 5 6
0 5 10 15 20
Geographic distance
f(elevation)
l= 0.45, p<0.001
0 1 2 3 4 5 6
0 1000 2500
Elevation
(b)
Plant driven interaction dissimilarity
1.00
0.75
0.50
0.25
0.00
ExpDev= 56.78, p<0.001
1 2 3 4 5 6
Ecological distance
f(geographic distance)
l= 0.30, p<0.001
0 1 2 3
0 5 10 15 20
Geographic distance
f(elevation)
l= 0.45, p<0.001
0 1 2 3
0 1000 2500
Elevation
(c)
Hummingbird driven interaction dissimilarity
1.00
0.75
0.50
0.25
0.00
ExpDev= 51.42, p<0.001
0 1 2 3 4 5
Ecological distance
f(geographic distance)
l= 0.44, p<0.001
0.0 1.0 2.0
0 5 10 15 20
Geographic distance
f(elevation)
l= 0.24, p<0.001
0.0 1.0 2.0
0 1000 2500
Elevation

2.11 TABLES

Table 1. Importance of each predictor and the explained deviance for the gdm full models, using the interaction turnover (βcc), the plant-driven interaction turnover (βpph) and the hummingbird-driven interaction turnover (βhhp) as response in each biogeographic unit. *Exp Dev* is the full model explained deviance. Importance is the proportion of the model deviance increase when each variable is dropped. Significant values of importance and explained deviance are in bold. Predictor labels: geo – geographic distance; temp – PCA axis for temperature variables; prec – PCA axis for precipitation variables; elev – elevation; chao – sampling completeness index. At the Chacoan dominion the geographic distance and elevation were removed from the analysis as they were strongly correlated to the precipitation and temperature respectively.

Biogeographical unit	Response variable	Exp Dev	Predictor importance				
			geo	temp	prec	elev	chao
Neotropical region	βcc	**69.86**	**0.59**	**0.02**	**0.01**	**0.06**	**0.03**
	βpph	**57.67**	**0.41**	0	**0.08**	**0.08**	0.01
	βhhp	**65.23**	**0.68**	**0.01**	**0.02**	**0.17**	**0.01**
Parana dominion	βcc	**69.20**	0	**0.27**	**0.48**	**0.23**	0.03
	βpph	**45.51**	0.01	0.02	**0.52**	0	0.02
	βhhp	**55.52**	0	**0.32**	**0.29**	**0.08**	**0.06**
Chacoan dominion	βcc	**59.85**	-	**0.44**	**0.34**	-	0.04
	βpph	**51.45**	-	**0.36**	**0.34**	-	0.03
	βhhp	**47.70**	-	**0.29**	**0.12**	-	0
Pacific dominion	βcc	**83.47**	**1.14**	0	0.02	**0.45**	0.15
	βpph	**56.78**	**0.30**	0	0.01	**0.45**	0.06
	βhhp	**51.42**	**0.44**	0	0.01	**0.24**	0.02

2.12 SUPPLEMENTARY MATERIAL

Table S1. Geographical coordinates and species richness of the 71 plant-hummingbird interaction networks.

Network ID	Latitude	Longitude	Hummingbird richness	Plant richness	Data source reference
1	-20.75	-42.92	8	14	Abreu, C.R.M. & Vieira, M.F. (2004). Os beija-flores e seus recursos florais em um fragmento florestal de Viçosa, sudeste brasileiro. Lundiana, 5, 129–134.
2	-23.35	-44.83	12	42	Araujo, A.C. (1996) Beija-flores e seus recursos florais numa área de planicie costeira do litoral norte de São Paulo, sudeste do Brasil. MSc. Thesis. Universidade Estadual de Campinas, Brazil.
3	-19.52	-56.98	4	13	Araujo, AC & Sazima, M (2003). The assemblage of flowers visited by hummingbirds in the "capões" of Southern Pantanal, Mato Grosso do Sul, Brazil. Flora, 198, 427–435
4	19.5	-105.05	5	15	Arizmendi, M.C. & Ornelas, J.F. (1990) Hummingbirds and their floral resources in a tropical dry forest in Mexico. Biotropica, 22, 172–180.
5	-20.51	-54.62	7	15	Barbosa-Filho, W. G., & Araujo, A. C. (2013). Flowers visited by hummingbirds in an urban Cerrado fragment, Mato Grosso do Sul, Brazil. Biota Neotropica, 13(4), 21-27.
6	-2.96	-79.1	9	19	Tinoco, B. A., Graham, C. H., Aguilar, J. M. and Schleuning, M. (2016), Effects of hummingbird morphology on specialization in pollination networks vary with resource availability. Oikos. doi: 10.1111/oik.02998
7	-2.87	-79.12	8	20	Tinoco, B. A., Graham, C. H., Aguilar, J. M. and Schleuning, M. (2016), Effects of hummingbird morphology on specialization in pollination networks vary with resource availability. Oikos. doi: 10.1111/oik.02998
8	-2.83	-79.13	12	19	Tinoco, B. A., Graham, C. H., Aguilar, J. M. and Schleuning, M. (2016), Effects of hummingbird

					morphology on specialization in pollination networks vary with resource availability. Oikos. doi: 10.1111/oik.02998
9	-31.8	-52.42	7	16	Vizentin-Bugoni, J. & Rui, A.M. Unpublished data.
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19	15.25	-61.37	2	11	Dalsgaard, B., Martín González, A.M., Olesen, J.M., Ollerton, J., Timmermann, A., Andersen, L.H. & Tossas, A.G. (2009) Plant–hummingbird interactions in the West Indies: floral specialisation gradients associated with environment and hummingbird size. Oecologia, 159, 757–766.
20	-23.33	-44.83	5	16	Maruyama, P.K, Vizentin-Bugoni, J., Dalsgaard, B., Sazima, I. & Sazima. M. (2015) Nectar robbery by a hermit hummingbird: association to floral phenotype and its influence on flowers and network structure. Oecologia, 178,783–793.
21	-23.36	-44.85	11	28	Maruyama, P.K, Vizentin-Bugoni, J., Dalsgaard, B., Sazima, I. & Sazima. M. (2015) Nectar robbery by a hermit hummingbird: association to floral phenotype and its influence on flowers and network structure. Oecologia, 178,783–793.
22	-24.56	-47.23	5	24	Fischer, E., unpublished data
23	12.1	-61.7	2	7	Dalsgaard, B., Martín González, A.M., Olesen, J.M., Ollerton, J., Timmermann, A., Andersen, L.H. & Tossas, A.G. (2009) Plant–hummingbird interactions in the West Indies: floral specialisation gradients associated with environment and hummingbird size. Oecologia, 159, 757–766.
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48	20.7	-98.77	2	9	Martínez-García, V., Ortiz-Pulido, R. (2014) Redes mutualistas colibrí-planta: comparación en dos escalas

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49	18.13	-66.76	2	11	Dalsgaard, B., Martín González, A.M., Olesen, J.M., Ollerton, J., Timmermann, A., Andersen, L.H. & Tossas, A.G. (2009) Plant–hummingbird interactions in the West Indies: floral specialisation gradients associated with environment and hummingbird size. Oecologia, 159, 757-766.
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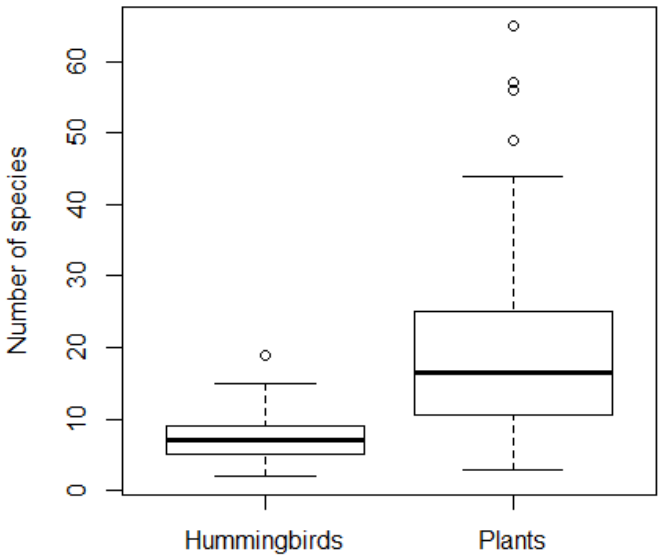
Table S2. For each biogeographic unit, the observed intercept (b.obs) and null intercept (b.null) for the models using as response variables the interaction turnover driven by plants (βpph) and by hummingbirds (βhhp). The null intercept is the mean intercept for 1000 rounds of the null model. We calculated intercept (b.obs and b.null) differences between the models using as response variables the interaction turnover driven by plants (βpph) and by hummingbirds (βhhp) (b. βpph - b. βhhp).

Biogeographical unit	Response variable	b.obs	b.null
Neotropical Region	βpph	0.70	0.96
	βhhp	0.05	0.87
	b. βpph - b. βhhp	0.65	0.08
Parana Dominion	βpph	0.76	0.75
	βhhp	0.00	0.54
	b. βpph - b. βhhp	0.76	0.21
Chacoan dominion	βpph	0.73	0.87
	βhhp	0.21	0.41
	b. βpph - b. βhhp	0.51	0.46
Pacific Dominion	βpph	0.46	0.88
	βhhp	0.08	0.71
	b. βpph - b. βhhp	0.38	0.16

Figure S1. Box plot showing the variation on plants and hummingbirds richness for the 71 interaction networks.



CAPÍTULO 2

DISENTANGLING THE EFFECT OF SPATIAL AND TEMPORAL SAMPLING EFFORTS ON PLANT-HUMMINGBIRD NETWORKS

3 CAPÍTULO 2 - DISENTANGLING THE EFFECT OF SPATIAL AND TEMPORAL SAMPLING EFFORTS ON PLANT-HUMMINGBIRD NETWORKS**

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3.1 SUMMARY

1. The observation of plant-pollinator interactions in the field is a challenge. Even highly sampled sites might show a severe incompleteness on its pollination interaction network. The temporal sampling effort is known to strongly influence the number of species and interactions included on the network as well as descriptive network metrics. Nonetheless, the importance of the spatial sampling effort has been less explored. The determination of the relative roles of temporal and spatial sampling effort might help on the design of more efficient sampling methods.

2.Our objective was to understand how the balance between the investment on spatial and temporal sampling effort affects the number of unique interactions, plants and pollinators detected on a plant-hummingbird interaction network. Moreover, we investigated how two network specialization metrics responded to the spatial and temporal sampling effort.

3. By using data from a well-sampled plant-hummingbird quantitative network, we simulated interaction samplings along increasing temporal and spatial sampling efforts. These simulations permitted the evaluation of the independent influence of temporal and spatial sampling efforts on the number of unique interactions and species, and on network specialization metrics (connectance and H_2').

4. The temporal sampling effort had a major role on the detection of plants and unique interactions. Although the spatial sampling effort had a minor influence, it enabled the observation of interactions on less common plants. The connectance showed a small decrease while the H2' was more stable along the spatial and temporal sampling effort gradient.

5. Our results reinforce the existence of strong sampling effects, primarily related to the time effort, when observing pollination interactions in the field. Furthermore, they emphasize the robustness of quantitative network structural descriptors. Our study contributes to the understanding of sampling effects by showing that the spatial sampling effort might be important in circumstances where the study site harbor many rare species. In this way, we forewarn about the necessity of carefully considering the spatial dimension of the sampling effects in pollination field works.

Keywords: Atlantic rainforest, Brazil, pollination, field methods, PPBio Mata Atlântica

3.2 INTRODUCTION

Field biologists often make use of sampling procedures to estimate biological parameters (Kenkel, Juhász-Nagy, & Podani, 1989). Nonetheless, choosing a suitable sampling design is a challenge. For plant-pollinator mutualism, the detection of a high proportion of the interactions in a community often demands an immense effort (Chacoff et al. 2012). Thus, understanding how sampling incompleteness of interaction data sets bias the conclusions of plant-pollinator interaction networks studies is of paramount relevance (Vázquez, Bluthgen, Cagnolo, & Chacoff, 2009).

A rising concern about sampling effects on interaction networks has led to several attempts to elucidate how networks features change because of the sampling effort. This was first explored in food webs, where studies revealed the existence of network metrics with different degrees of sensitivity to sampling effort (Banašek-Richter, Cattin, & Bersier, 2004; Goldwasser & Roughgarden, 1997; Martinez, Hawkins, Dawah, & Feifarek, 1999). More recently, studies showed that sampling effort might affect the perceived number of plants, pollinators and interactions, influencing plant-pollinator interaction networks architecture (Chacoff et al., 2012; Nielsen & Bascompte, 2007; Vizentin-Bugoni et al., 2016). In addition to that, there is a variable ability of different field sampling methods on detecting pollination interactions (Dorado, Vazquez, Stevani, & Chacoff, 2011; Gibson, Knott, Eberlein, & Memmott, 2011; Hegland, Dunne, Nielsen, & Memmott, 2010).

The sampling of pollination interactions is usually realized on the spatial and temporal dimension. Depending on how we increase the temporal sampling effort, networks will take account of interaction variability between years, seasons, days or hours (Alarcón, Waser, & Ollerton, 2008; Baldock, Memmott, Carlos Ruiz-Guajardo,

Roze, & Stone, 2011; Basilio, Medan, Torretta, & Bartoloni, 2006; Chacoff, Resasco, & Vázquez, 2018; Nielsen & Bascompte, 2007; Olesen, Bascompte, Elberling, & Jordano, 2008; Petanidou, Kallimanis, Tzanopoulos, Sgardelis, & Pantis, 2008). Thus, the amount of time spent observing flowering plant species, which is a common method to observe interactions, will have strong effects on the perceived interactions and network patterns (Rivera-Hutinel, Bustamante, Marin, & Medel, 2012; Vizentin-Bugoni et al., 2016). Species interactions also vary in the geographic space (Carstensen, Sabatino, Trøjelsgaard, & Morellato, 2014). In this way, increasing the spatial sampling also enhances the number of interactions observed. This might happen because of a spatial interaction turnover due to environment or habitat differences between the spatial samples (Maruyama, Vizentin-bugoni, Oliveira, Oliveira, & Dalsgaard, 2014; Simanonok & Burkle, 2014). In this cases, researchers might prefer to construct single networks for different habitats (Paine, 1988). Nonetheless, an increasing number of spatial samples might lead to increases on species richness just because of sampling effects (Colwell, Mao, & Chang, 2004). Because of sampling artifacts, rare species will be detected in fewer spatial samples (Gaston, Blackburn, & Lawton, 1997), and thus, by increasing the spatial effort, we increase the chance to record the presence of such species and its interactions. Although the relevance of spatial sampling effort in ecology (Colwell et al., 2004), studies evaluating the sampling effects on pollination networks have focused mainly on the time dimension. Nielsen & Bascompte(2007) observed that increasing the number of spatial samples in single habitats lead to increases on the number of links and species but not on the network nestedness. Nonetheless, their methodology combined the dual effect of spatial and time sampling effort, achieved when merging the data of different spatial samples. Thus, we still lack studies evaluating the influence of the spatial sampling effort independently of the time effort.

Different approaches are proposed to deal with sampling effects on pollination interaction networks, such as null models (Dormann, Frund, Bluthgen, & Gruber, 2009) and the use of less sampling sensitive network metrics (Blüthgen, Menzel, & Blüthgen, 2006). Additionally, the evaluation of sampling effects using empirical (Dorado et al., 2011; Gibson et al., 2011; Nielsen & Bascompte, 2007; Vizentin-Bugoni et al., 2016) or simulated data (Fründ, Mccann, & Williams, 2016) help to develop guidelines to reduce potential sampling biases. As field studies require a great investment of time and money (Hegland et al., 2010), understanding the consequences of sampling effects is crucial, not only to produce higher quality information, but also to permit more efficient ways to allocate resources (Hegland et al., 2010). Here our objective was to understand how the balance between the investment on spatial and temporal sampling effort affects the number of unique interactions, plants and hummingbirds detected on an Atlantic rainforest plant-hummingbird interaction network. Moreover, we investigated how two network specialization metrics responded to the spatial and temporal sampling effort. The environmental gradient and the spatial extent of the studied site are narrow. A recent study of of interaction beta diversity with plant-hummingbird interaction in the same area show that the turnover of interactions due to the turnover of plants and hummingbirds is low and not related to environmental or geographic distances between spatial samples (Bruno Reis *unpublished data*). Moreover, there is no correlation between the environmental distance and the geographical distance with plants and hummingbirds composition dissimilarity between the spatial samples (see Methods). Considering recent evidence of the role of the time spent observing plants on the detection of interactions with hummingbirds (Vizentin-Bugoni et al., 2016), we predicted an enhanced importance of the temporal sampling effort. Conversely, we

predicted that the spatial sampling effort would show a minor role, since we did not observed many rare plants and pollinators.

3.3 MATERIAL AND METHODS

Data collection

We collected interaction data in the Reserva Natural Guaricica at the Atlantic rainforest of Paraná (25°18'53.4"S 48°41'46.4"W), southern Brazil. From November 2014 to October 2015, we performed observations on 10 plots, separated 1km from each other. Each plot consists in 25 contiguous segments of 10 meters following a fixed altitudinal elevation, forming one big trail of 250 meters (central trail). We marked 20 meters perpendicularly from the central trail to have 25 rectangles of 10x20 meters (sub-plots) that together form a plot. This design is part of the RAPELD method (Magnusson et al., 2005) and is used on sites where research is being carried within the PPBio Mata Atlântica project of the Brazilian government (https://ppbio.inpa.gov.br). Within each plot, we searched for plant species with potential to have their flowers visited by hummingbirds. We observed each individual flowering plant found at each plot for ten minutes monthly, along 12 consecutive months. Every month two observers visited all the plots. While one observer started from the beginning of the central trail, the other started from the opposite end. Both walked along the sub-plots and when finding a plant, an observer would stand for 10 minutes recording hummingbirds - plant interactions, restarting the count every minute. Thus, each individual in a given month has 10 slices of one-minute observations. When all the individual plants had been sampled the observer passed to the next sub-plot. As the two observers would always go

in opposite directions, at some point they encountered each other, finishing the observation at a given plot. In this method of observation, abundant plants are observed for more time than rare plants (Rivera-Hutinel et al., 2012).

Using the interactions collected within the 10 plots, we constructed an interaction matrix between plants and hummingbirds species, where each cell corresponds to the number of times a hummingbird visited a plant species. We assessed the sampling completeness of this interaction network using a method proposed by Chacoff et al.(2012) and adapted by (Devoto, Bailey, Craze, & Memmott, 2012). This method consists in calculating the proportion of observed interaction richness relative to the maximum estimated interaction richness. To calculate the maximum interaction richness, we used the Chao 1 estimator in the estimateR function of the R vegan package (Oksanen et al., 2017). In this case, instead of providing information on the number of individuals of each species, we provided the amount of interaction of each pair of species. This method showed that we recorded 85% of the estimated interaction richness, and thus we consider the sampled network to be close to the real network, making it suitable to simulate the effects of sampling effects (Vizentin-Bugoni et al., 2016).

Correlation between species and ecological dissimilarity

We used Mantel matrix correlations to verify if the environmental and geographic distance were correlated to the plants and hummingbirds composition dissimilarity between the spatial samples (Legendre & Legendre, 1998). As environmental variables, we considered the altitude, the mean canopy cover and the mean declivity of the plots. These variables were used to construct an environmental

dissimilarity matrix. The plants composition dissimilarity was based on a matrix of plant species abundance on the plots. The composition dissimilarity of hummingbirds was based on a matrix containing each species interaction frequency in each plot (proxy for abundance) (Vázquez, Chacoff, & Cagnolo, 2009; Vizentin-Bugoni, Maruyama, & Sazima, 2014). The Mantel tests revealed that there is no correlation between the environmental or geographic distance and the plants or hummingbirds composition, suggesting that our spatial samples are located within a same relatively homogeneous habitat (Table S1).

Sampling effort simulations

With the purpose of disentangling spatial sampling effort from time sampling effort, we promoted simulations using the field data observations to create sampling effort gradients on the spatial and temporal dimensions. These sampling effort gradients were composed of classes of increasing number spatial or temporal units. The spatial sampling effort unit was each one of the ten plots observed at our study site. We defined the time sampling effort unit as the amount of minutes spent in focal observations on plant individuals along one year. We can visualize these sampling effort gradients in a triangular matrix, where we have increases of spatial sampling effort classes in a same time sampling effort class from left to right in a same row (Fig. 1). On the other hand, we have an increase of the time sampling effort class, in a same spatial sampling effort class, from the bottom to the top, in a same column (Fig. 1). Following diagonal lines, parallel to the triangle hypotenuse, we have an increase in both dimensions of the sampling effort (Fig. 1). To simulate interaction networks for each combination of

spatial and temporal sampling effort (each cell of the Fig. 1 matrix), we conducted the following steps:

1) Our field methodology lead to plant abundant sites to be observed for more time (observation minutes) across one year. Because of that, the first step was to standardize the time effort across all the ten plots. We did that by randomly sampling observation minutes of the plots until it matched the total effort of the least observed plot.
2) For a given temporal sampling effort class t_i and spatial sampling effort class s_j (Fig. 1), we simulated the merging of s_j plots with a t_i/s_j proportion of each plot observation minutes. For example, at the first temporal sampling effort class (t_1) and second spatial sampling effort class (s_2), we have t_1/s_2=½=0.5.Thus, we randomly reduced each of the 10 plots observation minutes to a half and used the interactions recorded in these 10 reduced sets of observation minutes to construct 10 interaction networks. Thereafter, we combined and merged these 10 networks in groups of 2, in all possible ways.
3) For each interaction network created in the step 2, we calculated different network metrics (see below).
4) We repeated the above 3 steps for 100 rounds.

After these four steps for a given spatial and temporal sampling effort class, we passed to the next combination of spatial and temporal sampling effort class (Fig. 1).

Network metrics

Here we used as network descriptors the richness of plants, hummingbirds and interactions in the network. Moreover, we assessed the specialization of the networks using two metrics, connectance and H_2' index. Although widely used to describe

network specialization, these metrics achieve that in different ways, reflecting different specialization concepts. Connectance is one of the most used metrics and it access the proportion of the realized interactions in relation to the possible interactions (Jordano, 1987). Regardless of its popularity, this metric is sensitive to sampling effort (Banašek-Richter et al., 2004; Blüthgen, Fründ, Vazquez, & Menzel, 2008; Blüthgen et al., 2006; Dormann, Frund, Bluthgen, & Gruber, 2009b; Fonseca & Ganade, 1996). Thus, with low sampling effort one network might appear specialized (low connectance) because low abundance species are subsampled, appearing to be specialiazed (Blüthgen, 2010). H_2' index measures network specialization by assessing how species interactions depart from what would be expected by interactions occurring based on species interaction frequency (Blüthgen et al., 2006). This measure has been shown to be robust to sampling effort (Blüthgen et al., 2008, 2006; Fründ et al., 2016; Vizentin-Bugoni et al., 2016). For each network descriptor, we constructed boxplots for each combination of temporal and spatial sampling effort (each cell of the Fig. 1 matrix).

We performed all the analysis within the R software (R Core Team, 2017) using bipartite (Dormann, Gruber, & Fründ, 2008) and vegan (Oksanen et al., 2017) packages, and *ad hoc* codes developed by the authors.

3.4 RESULTS

In ca. 553 hours of field observations, we recorded 551 interaction events, comprising 24 unique interactions between 4 hummingbirds and 13 plants. The treelet *Psychotria nuda* was abundant in all plots and received approximately 70% of the interactions (Table 1). *Thalurania glaucopis* and *Ramphodon naevius* were the

predominant hummingbirds, comprising 55% and 36% of all interactions (Table 1). Most plant species were observed flowering in five or more plots, but some plants were observed flowering in just a few or even just one plot (Table 1). In addition, the number of plots a plant species was recorded flowering was strongly correlated to its abundance in the entire study site (abundance of flowering individuals;$r=0.91$, $P<0.001$). Three hummingbird species were observed interacting in seven or more plots, while one species was detected interacting with plants in two plots (Table 1).

Increasing the spatial sampling effort, while maintaining the temporal sampling effort, caused mild effects on the plant richness of the simulated networks (Fig. 2a). At the two first temporal sampling effort classes (t_1 and t_2), the number of interacting plant species tended to increase with discrete increases on the number of spatial samples (Fig. 2a). This occurred because of a higher number of plant species available for observation with more spatial samples (Fig. 3). Indeed, by combining just three plots, there is the possibility of observing a mean of 11 plant species, which represents 85% of the total interacting plant richness at the study site (Fig. 3). Nonetheless, only increasing the number of plots was not sufficient to allow the inclusion of all of these plants in the network. Indeed, we observed that within each spatial sampling effort class, increases on the temporal sampling effort lead to constant increases on the number of interacting plants, until about seven-fold increases (Fig. 2a). After merging three plots, further increases on the spatial effort, without increasing the temporal effort, did not reveal more plants in the network, even causing a decrease tendency at higher spatial sampling effort classes (Fig. 2a). This decrease tendency at high spatial efforts occurs in high temporal effort classes as well (Fig. 2a). The median of 12 plant species at the simulated highest sampling effort networks ($t_{10}s_{10}$) was slightly lower than the maximum found at our field sampling (13 interacting plant species). This is a result of the first step in our

simulation procedures, where we reduced the time of all plots to standardize the temporal effort across plots, leading to a small loss on the number of plant species in the network.

Along the first four spatial sampling effort classes, there was a tendency to reach the total hummingbird richness (Fig. 2b). In these spatial sampling effort classes, increasing the time effort did not help to detect the complete hummingbird richness (Fig. 2b). After five plots merged, the detection of interacting hummingbirds became a matter of enhancing the temporal sampling effort (Fig. 2b).

Similar to the number of plant species at the network, for low time sampling efforts classes, the number of unique interactions showed a tendency of increase with small increases on the number of plots, followed by a stabilization after that and even small decreases at higher spatial sampling effort classes (Fig. 2c). By looking within each spatial sampling effort, we detected the temporal sampling effort as an important factor for including new links at the network (Fig. 2c). With five spatial samples, all plant and hummingbird species start to be frequently available for interactions (Fig. 3), what means that after an intermediate spatial sampling effort only the increase on temporal effort will detect more unique interactions. The median number of unique interactions at the higher simulated sampling effort (21 unique interactions) was lower than the maximum observed at the study area (24 unique interactions). Once again, we identify this difference as a result of the simulated reduction of the total observation across plots to standardize the total temporal effort.

Increasing the spatial and the temporal effort tended to reveal less connected networks at the beginning of both gradients (Fig. 4a). The highest sampling effort ($t_{10}s_{10}$) revealed networks with intermediate connectance (Fig. 4a).

The H_2' index did not show any decreasing or increasing trend along the sampling effort gradient, assuming values under 0.5 for the majority of the simulated networks (Fig. 4b). Nonetheless, this index showed considerable variation when the effort at the time intensity was low (Fig. 4b). Moreover, in some cases, at very low sampling efforts, the reduction on the number of pollinators and plants was so drastic that the interaction network was composed of just one plant or hummingbird. At these cases, the H_2' index could not be calculated, and this networks were excluded from the boxplot.

3.4 DISCUSSION

Our results reinforce the importance of the temporal sampling effort for the perception of pollination interactions in biological communities. Furthermore, we showed that spatial sampling effort might also be determinant, mainly in sites that present many rare species. In a tropical rainforest habitat, the time spent looking for interactions was crucial, while the spatial effort presented a relatively smaller role. These results depict the importance of disentangling different dimensions of the sampling effort, what might guide the sampling design and resources allocation in field studies (Saito, Fonseca-Gessner, & Siqueira, 2015).

The detection of uncommon objects in a statistical population requires high sampling efforts (Kalton & Anderson, 1986). Such characteristic will cause a tendency of low abundance species to be detected in fewer spatial locations, just by sampling artifacts (Gaston et al., 1997). Indeed, the strong correlation between flowering plant species abundance and the number of plots where they were detected in our study area

is an evidence of that. In pollination studies focused on plant focal observations, such sampling artifact will be more problematic when a rare plant species has also a small flowering period. Our analysis reveal that small increases on spatial sampling effort are beneficial, by giving the opportunity to observe less common plant species. After having the opportunity to observe these species, time investment is necessary to observe interactions (Vizentin-Bugoni et al., 2016). The occurrence of different interactions in a plant will also depend on pollinator's presence at the same spatial location (Vázquez, Chacoff, et al., 2009). Nevertheless, the low diversity of hummingbirds at our study site, and the fact that most species occurred at most of the plots, means that the effect of the spatial sampling effort on the richness of interactions is more restricted to the richness of plants. This is reflected on the plant and interaction richness similar trends along the sampling effort gradients. Still, the low occurrence of one hummingbird species (*Aphantochroa cirrochloris),* that is more common in open habitats or at forest edges (Ridgely, Gwyne, Tudor, & Argel, 2015), further enhance the need of spatial samples.

At our study site, the most efficient way to sample would be to invest in less sample plots but with higher temporal effort. Thus, our original sampling design could be improved by sampling five spatial samples, for example, and staying for more than ten minutes observing each individual plant. In this way, we would give the chance to include interactions between all plants and hummingbirds, while reducing the number of days in field and restricting the movement of researchers across the study area. The network we observed in our fieldwork had high sampling completeness but certainly still suffered from some incompleteness. This incompleteness, even in a high sampling effort, might be related to the observation unevenness across plant species (Rivera-Hutinel et al., 2012), what may lead to the impression that rare species are specialists, even when they are not, biasing network descriptors, particularly specialization metrics

(Blüthgen et al., 2008; Vázquez & Aizen, 2003). Thus, another possibility to improve our original sampling method would be to stay for ten minutes observing each individual and, after observing all individuals, visit the individuals of the less abundant plants for more time.

The tendency of connectance to decrease along the sampling effort is related to a high rate of new interactions possibilities, caused by the inclusion of new species in the network, paralleled by a lower rate of formation of new interactions (Goldwasser & Roughgarden, 1997; Nielsen & Bascompte, 2007; Vizentin-Bugoni et al., 2016). Nonetheless, our site diversity of hummingbirds and plants is low when compared to other studies in the Atlantic rainforest (Buzato, Sazima, & Sazima, 2000; Vizentin-Bugoni et al., 2014), leading to a limited decrease on the connectance along the sampling effort gradient. As expected, the H_2' index is more robust than the connectance, reinforcing that quantitative metrics are generally less affected by sampling effects than binary ones (Blüthgen et al., 2006; Fründ et al., 2016; Vizentin-Bugoni et al., 2016). Nonetheless, as previously detected, poorly sampled networks leads to an instability on the specialization metrics values (Blüthgen et al., 2006; Vizentin-Bugoni et al., 2016), showing that even robust metrics might lead to wrong conclusions in extreme cases of sampling incompleteness.

3.6 CONCLUSIONS

Here we reinforced the strong sampling effects when sampling pollination networks, primarily related to the time effort. Nonetheless, we moved forward, showing

that the spatial sampling effort might be strong in some circumstances, especially when there are many rare species on a study area.

The interpretation of our results evidenced that, for a single relatively homogeneous habitat, investing more time in fewer plots is likely to be more efficient than spreading the time effort across many spatial samples. This enables a more efficient use of resources, which might be partitioned to other research projects. For example, Saito et al.(2015), after investigating the efficiency of their sampling on fish species in streams, concluded that they could have reduced their spatial sampling within each stream, what would have liberated time and money to sample even more streams. This would have increased their study extent and possibly its power of generalization. Our results are important in the context of the ongoing Brazilian PPBio Mata Atlântica project. This study is the first to access plant-pollinator interactions at this project and our results might guide sampling planning in other sites of the same project. Moreover, our simulation methodology could be refined to receive preliminary interaction data, giving researchers an idea of the best way to balance the temporal and spatial sampling effort.

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3.9 TABLES

Table 1. Plant and hummingbird species that interacted in the study site along the period of our fieldwork. Labels: Interactions (%) = percentage of the interactions realized by each species in the study site; Plots (n) = number of plots where each plant species was registered in flower and each hummingbird species was registered realizing interactions; Abundance = number of flowering plant species individuals and the interaction frequency for hummingbirds (proxy for abundance) in the study site

Species	**Interactions (%)**	**Plots (n)**	**Abundance**
Plants			
Aechmea nudicaulis	34	7	29
Aechmea ornata	0.4	1	2
Costus spiralis	2.2	9	46
Dahlstedtia pentaphylla	0.5	5	15
Heliconia farinosa	0.9	2	5
Musa ornata	1.9	5	50
Nidularium innocentii	12.7	10	368
Nidularium procerum	1.1	3	10
Psychotria nuda	70.5	10	1851
Psychotria suterella	2.2	9	87
Spirotheca rivieri	0.4	5	6
Vriesea carinata	1.4	10	543
Vriesea incurvata	3.1	10	218
Hummingbirds			
Amazilia versicolor	4.7	7	26
Aphantochroa cirrochloris	4.2	2	23
Ramphodon naevius	36.3	10	201
Thalurania glaucopis	54.8	10	301

3.10 FIGURES

Figure 1- Triangular matrix showing the combinations of spatial (x-axis) and temporal (y-axis) sampling effort classes (s_j and t_i). For each combination, we performed sampling effort simulations. The colors at each row are the same used to represent each temporal sampling effort at the results boxplots (Figs. 2 and 4).

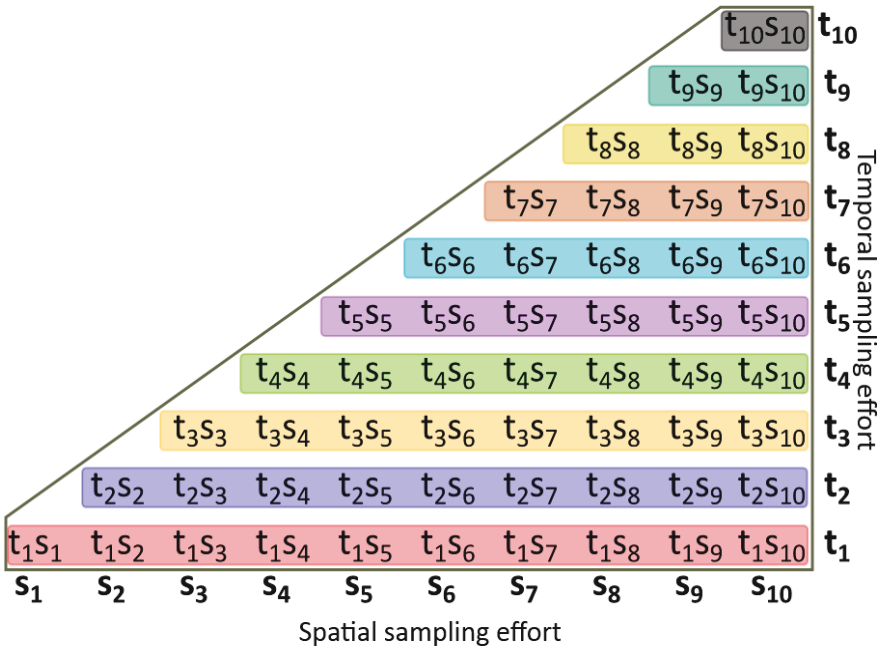


Figure 2. Boxplots showing the richness of plants (a), hummingbirds (b) and the number of unique links (c) across the combinations of spatial (s_j) and temporal (t_i) sampling effort classes. Each colour represents a temporal sampling effort class (the same colours used in the Figure 1). Following boxplots of a same colour from left to right describes the variation of each network descriptor along increasing spatial sampling effort classes in a same temporal effort class. Following boxplots within a same spatial sampling effort class, from the bottom to the top, describes the variation of each network descriptor along increasing temporal sampling effort classes.

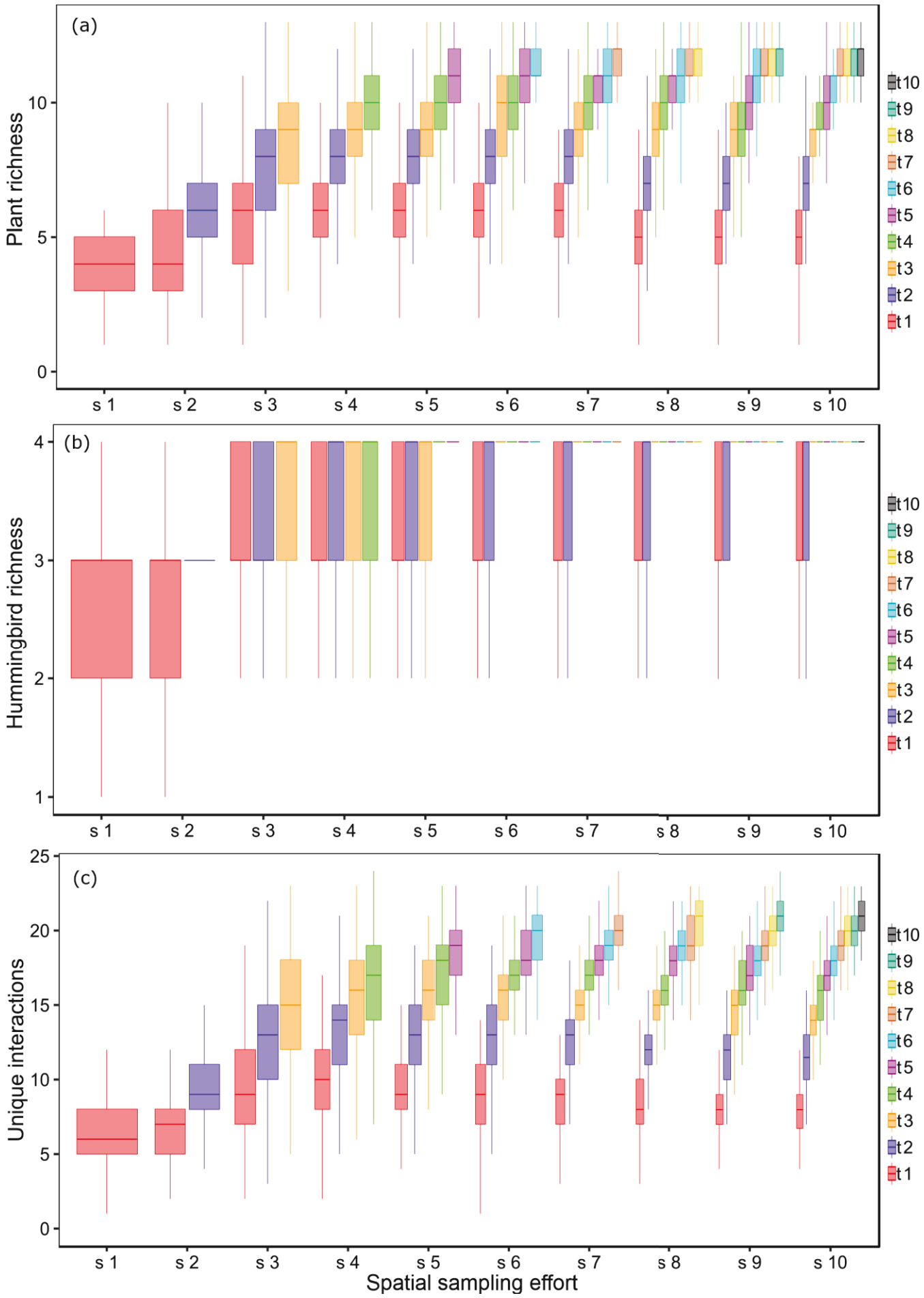

(a)
Plant richness
0
5
10
(b)
Hummingbird richness
1
2
3
4
(c)
Unique interactions
0
5
10
15
20
25
s 1
s 2
s 3
s 4
s 5
s 6
s 7
s 8
s 9
s 10
Spatial sampling effort
t10
t9
t8
t7
t6
t5
t4
t3
t2
t1

Figure 3. Mean richness of flowering plant species (filled circles) and hummingbird species (empty circles) that would be available for being detected performing interactions, when merging different number of plots. Note that by merging only three or four plots, most of the plants are already available for observations.

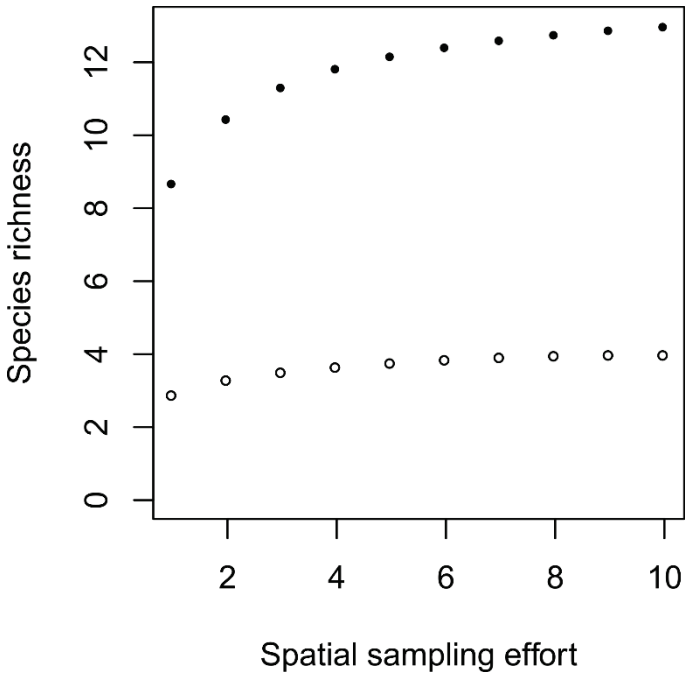
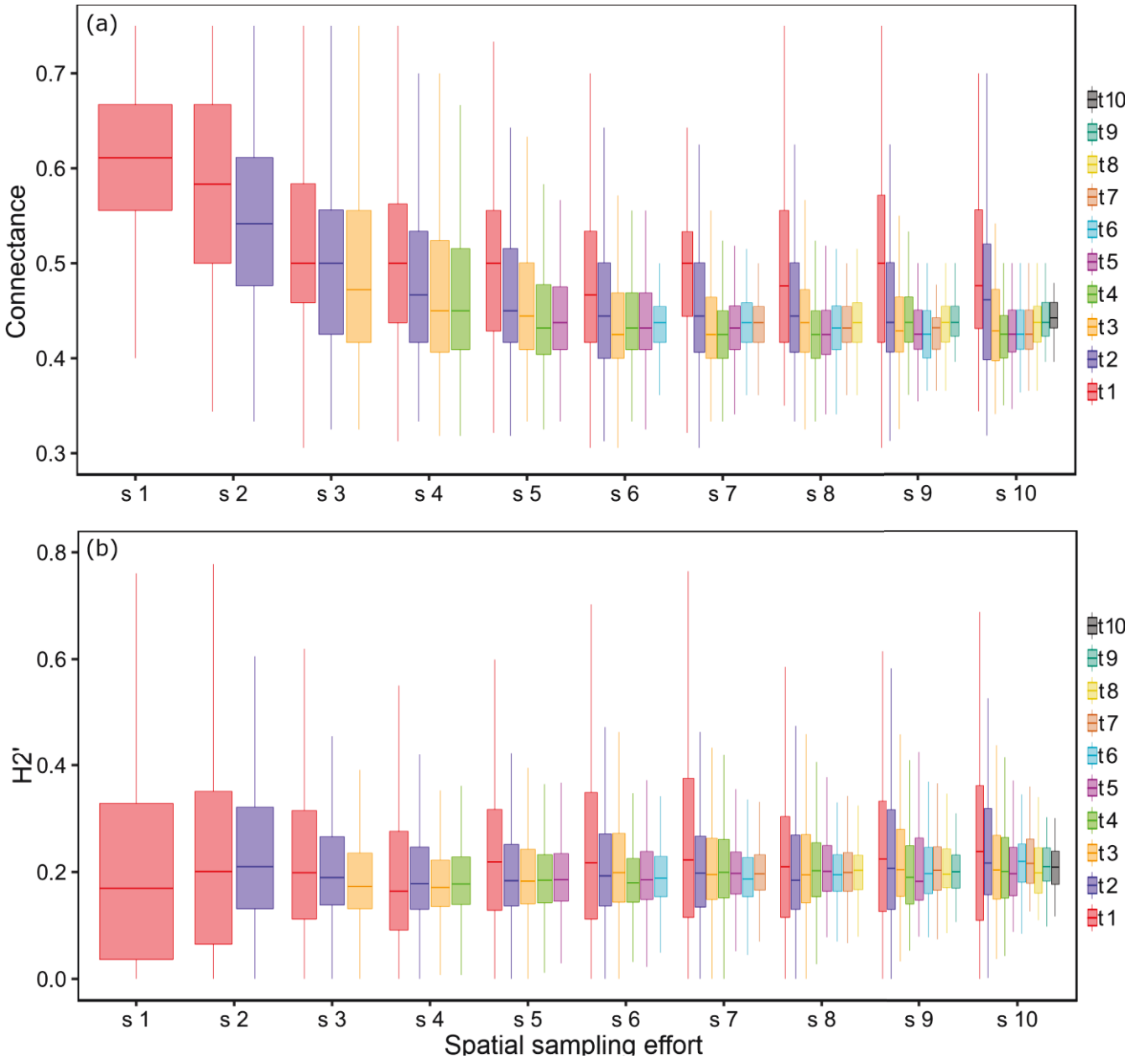


Figure 4. Boxplots showing connectance (a) and *H_2'* index (c) values across the combinations of spatial and temporal sampling effort classes. Each colour represents a temporal sampling effort class (the same colours used in the Figure 1) Following boxplots of a same colour from left to right describes the variation of each network descriptor along increasing spatial sampling effort classes in a same temporal effort class. Following boxplots within a same spatial sampling effort class, from the bottom to the top, describes the variation of each network descriptor along increasing temporal sampling effort classes.



3.11 SUPPORTING INFORMATION

Table S1. Mantel correlation between plants and hummingbirds dissimilarity with the environmental and geographic distance. The coefficient of correlation is represented by *r*. None of the correlations were significant ($p<0.05$).

	Plant dissimilarity		**Hummingbird dissimilarity**	
	r	*p*	*r*	*p*
Environmental distance	0.53	0.11	0.23	0.18
Geographic distance	0.29	0.06	0.24	0.07

CAPÍTULO 3

PLANTS WITH RESTRICTIVE POLLINATION TRAITS ARE ASSOCIATED TO AN ENHANCED RISK OF INTERACTION DECLINE IN A PLANT-HUMMINGBIRD NETWORK SUBMITTED TO RANDOM PERTURBATIONS

4 CAPÍTULO 3 - PLANTS WITH RESTRICTIVE POLLINATION TRAITS ARE ASSOCIATED TO AN ENHANCED RISK OF INTERACTION DECLINE IN A PLANT-HUMMINGBIRD NETWORK SUBMITTED TO RANDOM PERTURBATIONS***

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4.1 ABSTRACT

The processes that structure plant-hummingbird pollination networks are driven by a combination of niche- and neutral/abundance-based factors. Natural or anthropic perturbations that affect these drivers might lead to changes on the structure of these networks. Here our aim was to understand, in scenarios where the interaction drivers were perturbed, if the maintenance of morphological and phenological interaction drivers unperturbed would reduce the overall modification on the distribution of interaction probabilities within the network. Moreover, we aimed to understand how species interaction probabilities would change when maintaining these drivers unperturbed. We investigated that by using data of an empirical plant-hummingbird interaction network that is well predicted by neutral, morphological and phenological drivers. We used species abundances and traits to create an interaction probabilistic model based on these three drivers. We simulated in this probabilistic model, random perturbations on all interaction drivers at a same time and we compared these simulations to ones where we kept the morphological or the phenological coupling driver unperturbed. Our results have shown contrasting consequences. In one hand, the maintenance of trait-based drivers reduced the overall change on the interactions probability distribution within the network. Nevertheless, for plant species that show restrictive traits (long corolla or short flowering period), there was an enhanced risk of reducing their interaction probability. For plants that were restricted by long corollas, this reduction frequently led to their exclusion from the network.

Keywords: Atlantic rainforest; Brazil; pollination interactions, interaction probability, interaction drivers.

4.2 INTRODUCTION

Plant-pollinator mutualistic interactions have always fascinated biologists (Bronstein 1994). Particularly in last decades, with the aid of the complex networks theory, we have experienced an explosion of studies exploring these interactions in a community perspective (Heleno et al. 2014). This led to the proposal of general structural patterns (Bascompte et al. 2003, 2006, Vázquez and Aizen 2004, Olesen et al. 2007, Vázquez et al. 2007) and to the identification of pollination interaction drivers (Stang et al. 2006, Vázquez et al. 2009b, Maruyama et al. 2014, Vizentin-Bugoni et al. 2014). Understanding how perturbations on drivers of pollination interactions affect the capacity of species to persist in natural communities is an important step to predict the consequences of the intense anthropic pressures on pollination networks (Memmott et al. 2007, Hegland et al. 2009).

It is widely known that niche-based process are involved on the structuration of pollination networks, where morphological and phenological species traits play important roles. Pollination morphological traits, might drive interactions by creating barriers that prevent some interactions from occurring (Jordano 2003, Stang et al. 2006, Santamaría and Rodríguez-Gironés 2007, Geerts and Pauw 2012, Maruyama et al. 2014, Vizentin-Bugoni et al. 2014), or by trait matching (Santamaría and Rodríguez-Gironés 2007, Stang et al. 2009). In addition, phenological traits, such as the extent of the flowering period of plants and migratory patterns of pollinators, constrain the availability of these organisms on the time dimension, indirectly contributing to the determination of which and how much species will interact (Vázquez et al. 2009b, Maruyama et al. 2014, Vizentin-Bugoni et al. 2014). A contrasting view to niche-based interaction drivers is that species interactions and network structure might arise also

because of species chances of encounters, depending on their abundances (Vázquez et al. 2007, 2009b, Krishna et al. 2008). Evidence has pointed for the combined action of neutral- and niche-based factors on the determination of pollination interactions and the network structure, with variable importance depending on the system (Stang et al. 2006, 2007, Vázquez et al. 2009b, Vizentin-Bugoni 2017).

Although species traits and abundances are closely related to the establishment of interactions, they are influenced by other factors that interact in complex ways (Vázquez et al. 2009a, Bartomeus et al. 2016). Thus, natural or anthropic perturbations that affect species abundance and/or traits might change how species interact in a community (Memmott et al. 2007, Hegland et al. 2009). Abundance fluctuations are ubiquitous in biological communities, and might be driven by natural (e.g. Wolda 1978, Peterson 2000) and anthropic perturbations (e.g. Wallace et al. 2017). These perturbations will indirectly affect species chances of encounters and their probability of interactions. Severe cases of abundance reduction of plants or pollinators might cause a cascade effect of negative consequences for other species in pollination networks (Memmott et al. 2004, Kaiser-Bunbury et al. 2010, Traveset et al. 2017). Species traits might vary in response to external factors as well, what might potentially disrupt interactions. For example, several plant species have been changing their flowering period in response to the climate warming (Fitter et al. 1995, Shi et al. 2014), what might lead to negative consequences for their pollinators due to temporal mismatches, especially for the specialists (Memmott et al. 2007).

Once perturbations on interaction drivers affect the probability of interactions between species, negative consequences for some of them might be compensated by different mechanisms. One important mechanism, on which species rely, are rapid behavioral modifications that permit species to change partners, reducing the risk of

extinction (Kaiser-Bunbury et al. 2010, Timóteo et al. 2016). Nonetheless, this possibility will be low for species that are constrained by specializations on their pollination-related traits, increasing the risk of extinction (Stang et al. 2007). In this case, evolutionary changes on species traits might help species to persist (Smith et al. 1995, Bodbyl and Kelly 2011). Nevertheless, these changes might occur in a large evolutionary time scale, what could be insufficient to allow species persistence to rapid modifications on interaction frequencies (Aitken et al. 2008). Besides that, phenotypic plasticity of some traits might help species to persist (Ghalambor et al. 2007), although the phenotypic plasticity will not necessarily lead to advantageous modifications (Hendry 2015).

In this context, our objective was to understand if, in scenarios of perturbation on the interaction drivers, maintaining morphological or phenological interaction drivers constant would reduce the overall modification on the distribution of interaction probabilities across the interaction matrix. Furthermore, we aimed to understand how the species interaction probability would change, and possibly decline, when maintaining these drivers unperturbed. We investigated that by using data of an empirical plant-hummingbird interaction network that is well predicted by neutral, morphological and phenological drivers. We used species abundances and trait data to create an interaction probabilistic model based on neutral, morphological and phenological drivers. We simulated, in this probabilistic model, random perturbations on all interaction drivers at a same time and compared to simulations where we kept the morphological or the phenological coupling drivers unperturbed. We predicted that maintaining morphological and phenological drivers without perturbation would reduce the overall change on the distribution of probabilities across the interaction matrix.

Nonetheless, we predicted that this would cause a decline on the interaction probability of species with restrictive morphological pollination traits (Stang et al. 2007).

4.3 MATERIAL AND METHODS

Data collection

We collected interaction data in the Reserva Natural Guaricica at the Atlantic rainforest of Paraná (25°18'53.4"S - 48°41'46.4"W), southern Brazil. From November 2014 to October 2015, we performed observations on 10 plots, spaced by 1km. Each plot consists in 25 contiguous segments of 10 meters following a fixed altitudinal elevation, forming one big trail of 250 meters (central trail). We marked 20 meters perpendicularly from the central trail to have 25 rectangles of 10x20 meters (sub-plots) that together form a plot. This kind of design is part of the RAPELD method (Magnusson et al. 2005) and is used on sites where research is being carried within the PPBio Mata Atlântica project, of the Brazilian government (https://ppbio.inpa.gov.br). Within each plot, we searched for plant species with potential to have their flowers visited by hummingbirds. We observed each individual flowering plant found, at each plot, for ten minutes monthly, along 12 consecutive months. Every month two observers visited all the plots. While one observer started from the beginning of the central trail the other started from the opposite end. Both walked along the sub-plots and when finding a plant, an observer would stand for 10 consecutive one-minute periods recording hummingbird-plant interactions, restarting the count every minute. When all the individual plants had been sampled, the observer passed to the next sub-plot. As the two observers would always go in opposite directions, at some point they encountered

each other, finishing the observation at a given plot. In this method of observation, abundant plants are observed for more time than rare plants (Rivera-Hutinel et al. 2012). Data of interactions observations are deposited at the MetaCat repository (https://ppbiodata.inpa.gov.br/metacatui/#view/PPBioMA.35.3). Using the interactions collected within the 10 plots, we constructed an interaction matrix between plants and hummingbirds species, where each cell corresponds to the number of times each pair of species interacted. This matrix was normalized to sum one, resulting in an observed interaction frequency matrix (O).

Probabilistic matrices

We constructed matrices of interaction probabilities (probabilistic matrices) (Fig. S1) to assess the importance of morphological barriers, phenological coupling and species abundance, as well as the combination of them, as drivers of the observed pairwise frequency of interactions. Such approach has been widely used for this purpose in pollination networks (Vázquez et al. 2009b, Maruyama et al. 2014, Vizentin-Bugoni et al. 2014).We constructed the abundance probabilistic matrix (A; Fig. S1a) based on the multiplication of plant species flower abundance by hummingbird species abundance. The flower abundance was assessed by counting all the flowers of each species along one year of observations on the 10 plots (Table 1). We considered hummingbirds interaction frequencies as a proxy of their abundances (Table 1; Vázquez et al. 2009b, Maruyama et al. 2014, Vizentin-Bugoni et al. 2014). In this matrix, the magnitude of each cell value represents the relative probability of a pair of species to interact by random encounters, determined by their abundances. We constructed the phenological-coupling probabilistic matrix (P; Fig. S1b) based on multiplications of the temporal-occurrence matrix of hummingbirds by plants flowering-period matrix, at a

month resolution (Table 1). This resulted in a plant-hummingbird matrix where cell values represents the relative probability of a pair of species to interact based on their temporal overlap. As the four hummingbirds are resident, occurring in all months this matrix do not show forbidden links created by temporal mismatches. To produce the morphological barrier-probability matrix (M; Fig. S1c), we compared hummingbird's bill length (Grantsau 1988) and plants corolla depth (Table 1). To account for the tongue extension (Vizentin-Bugoni et al. 2014), we considered that each hummingbird species can extend the tongue to a same length of the bill. We accessed corolla depth by calculating the mean depth of at least ten flowers of each plant species (Table 1). For a pair of species where the two-fold bill length (i.e. measurement including the length of the tongue) was equal to or greater than the corolla depth, the interaction was considered possible, i.e. no morphological barrier, and received a value of one on the morphological barriers probabilistic matrix. If this condition was not satisfied, the interaction was considered impossible due to morphological barriers, and received a value of zero. For three species of plants (*Musa ornata*, *Nidularium innocentii* and *Nidularium procerum*), we observed several interactions with hummingbirds species that, following our morphological constraint rule, should not happen. We reduced the corolla constraint for these species to match the bill and tongue extension of the unexpected hummingbird's visitors (Maruyama et al. 2014). Additionally, we constructed a null probabilistic matrix (null) where all interactions received the same probability value. All these single probabilistic matrices (A, P, M, null) were normalized to sum one. The combinations of PA, MA, MP and MPA was done, by multiplying each cell of a probabilistic matrix by the respective cell of another probabilistic matrix. After normalizing these combined probabilistic matrices, we ended with eight probabilistic models (M, P, A, PA, MA, MP, MPA and null). The value in

each cell of these probabilistic models represents the probability of an interaction based on a given driver or on combined drivers.

Similarity between the probabilistic and observed matrices

In order to determine the power of each probabilistic model to predict the observed interaction frequencies, we assessed the similarity of each probabilistic model R = {M, P, A, PA, MA, MP,MPA, null} to the observed interaction frequency matrix O. This was done by calculating an index of similarity between matrices. This index takes in account the sum of the absolute difference between the cells of each probabilistic model (*Rij)* and the observed interaction frequency matrix (*Oij*).

$$s = 1 - \frac{1}{2}\sum_{ij}(|Rij - Oij|) \quad (1)$$

The hypothetical maximum value of the term $\sum_{ij}(|Rij - Oij|)$ is two. Thus, the term ½ is a normalization parameter that limits "*s"* between zero and one. If the value of similarity (*s*) tends to zero, it means that the cells of the probabilistic models and the observed interaction frequency matrix are completely dissimilar. If the value of similarity (*s*) is equal to one, the elements of the probabilistic model matrix fits perfectly the observed interaction frequency matrix. We have found that the model combining the three drivers (MPA, Fig. S1) was the most similar to the observed interactions frequency matrix, with *s* = 0.86 (Table 2). Thus, we used this probabilistic model to perform simulations of perturbations on the interaction drivers (see below).

Perturbation of the probabilistic matrices

We simulated perturbations on the MPA probabilistic model by perturbing with increasing intensities the individual probabilistic matrices (M, P and A) that composes the MPA model. In other words, we simulated perturbations on the interaction drivers. We first simulated increasing intensity perturbations on the three drivers (MpPpAp; "*p*" denotes which interaction driver was perturbed). As our goal was to understand the effects of maintaining morphological or phenological drivers unperturbed, we simulated perturbations on the MPA model but keeping either the morphological driver (MPpAp) or the phenological driver (MpPAp) unperturbed. These simulations (MPpAp and MpPAp), might be interpreted as perturbation situations where species cannot modify their traits. Our expectation was that random perturbations would mean negative consequences more frequently for species that are restricted by their pollination traits than for species that are not restricted by these traits.

To create perturbation on the drivers, we added random values to each cell of the individual probabilistic matrices (M, P or A) from the MPA model, that needed to be perturbed. The random value correspond to a value drawn from a normal distribution with mean zero and standard deviation σ. After adding random values to an individual probabilistic matrix, cells that received a negative value were updated to zero, because a negative probability of interaction would be meaningless. Thereafter, we normalized the matrix to sum one. To create the perturbed probabilistic models (MpPpAp, MPpAp, MpPAp) from the individual probabilistic matrices, we multiplied the three individual probabilistic matrices, and normalized to sum one. As we wanted to simulate a gradient of increasing perturbation intensity, we used normal distributions with σ ranging from 0 to 0.5 by increases of 0.005. For each perturbation intensity (σ), we replicated the above procedures 3000 times.

For each perturbed probabilistic model (MpPpAp, MPpAp, MpPAp) in a given perturbation intensity (σ), we recalculated the similarity to the observed interaction frequency matrix. Thus, we could assess for each perturbed probabilistic model how much and how fast it departed from the original, unperturbed condition, along the gradient of increasing perturbation intensity. Moreover, we calculated the probability of interaction of each individual species, what corresponds to the sum across columns/rows of the perturbed probabilistic models matrices.

With the species interaction probability values for the perturbed models, we calculated, for each species, how many times perturbations led to decreases on their original interaction probability (their probability on the MPA model). In addition, we calculated how many times this decrease led to zero interaction probability. With this we could assess the chance of species to face negative consequences (reduction on the interaction probability) related to the perturbation on different interaction drivers. All the analysis were realized in the R software (R Core Team, 2016).

4.4 RESULTS

All of the perturbed probabilistic models showed a fast similarity decay with perturbation intensity, followed by a stabilization (Fig. 1). Nevertheless, we detected that the models maintaining either M or P unperturbed, MPpAp and MpPAp probabilistic models, presented a similar decay, with a less intense decay than the MpPpAp probabilistic model, where all drivers were perturbed (Fig. 1).

The perturbation of all of the interaction drivers (model MpPpAp) showed that the interaction probability between plants and between hummingbirds tended, on average, to converge to a same value with the perturbation intensity (Fig. 2). This

convergence is due to a mathematical effect of our method: when a species has a low interaction probability on the MPA model, perturbations on this species matrix cells, in many cases, results in negative values. As these negative values are updated to zero (once negative probability has no sense, as we mentioned before, the average probability of interactions for this species tend to increase. This implies that when all drivers are perturbed, there is a tendency to simulate an increase of interaction probabilities for species with lower initial interaction probabilities and to simulate a decrease of interaction probabilities for species with higher initial interaction probabilities (Fig. 2).

Maintaining the morphological barriers or the phenological coupling drivers unperturbed reduced the tendency of convergence reported before, for some species (Fig. 3, S2 and S3). Specifically for two low interaction probability plant species, which present long corollas (*Costus spirarlis* and *Vriesea carinata*; Table 1) that restrict their number of partners to one hummingbird (Fig. S1c, d); maintaining the morphological barriers driver unperturbed (MPpAp model) reduced the mean interaction probability increase (Fig. 3). This was caused by the fact that many perturbations, which in the model MpPpAp would lead to increases on the interaction probability, were blocked by morphological forbidden links in the MPpAp model. More specifically, this occurs because perturbations on P and A, that happened to increase the probability of these species to interact to the four species of hummingbirds, were multiplied by three cells containing zeros in M, that represents the three morphologically forbidden interactions with hummingbirds. Moreover, these species showed an enhanced chance of having its interaction probability reduced and an enhanced chance of this reduction leading to a zero interaction probability, when compared to the model MpPpAp (Table 3). On contrary, when we only fixed the phenological coupling driver (model MpPAp), the

interaction probability tendency was to increase in a similar way to the MpPpAp model (Fig. 3), as both species were not restricted in their flowering period, i.e. long flowering period (Table 1). Moreover, fixing the phenological coupling driver did not increased the chance of reducing the interaction probability of these species, when compared to the model where no interaction driver was fixed (model MpPpAp; Table 3).

There was a positive correlation between plant species flowering period length and their interaction probability on the MPA model ($r=0.65$, $p<0.05$). This show that very short flowering period were restricted by this trait. For plant species that had short flowering periods (Table 1) and, consequently, low interaction probabilities, maintaining the phenological coupling matrix unperturbed (model MpPAp) reduced the mean interaction probability increase (Fig. S2). In the Figure 3, we depict two examples of this situation, with plant species that showed a reduced flowering period (*Aechmea nudicaulis* and *N. procerum*; Table 1). For both species, the reduced tendency of increase on the mean interaction probability was caused by the fact that many perturbations, which in the model MpPpAp would lead to increases on the interaction probability, were not incorporated on the MpPAp model (Fig. 3). This occurs because perturbations on M and A, that happened to increase the probability of these species to interact to hummingbirds, were multiplied by very low values in P, which represents the low chance of these species to interact due to a short occurrence on the time dimension. On contrary, when the morphological barriers were kept unperturbed, the tendency was to follow the increase on the probability seen at the model MpPpAp (Fig. 3), as these species have relatively short corollas (Table 1). *Nidularium procerum* did not show an enhanced chance of having its interaction probability reduced when maintaining the phenological coupling driver unperturbed (MpPAp model), in comparison to the MpPpAp model (Table 3). *Aechmea nudicaulis* showed a mild increase on the chance of

having its interaction probability reduced when maintaining the phenological coupling driver unperturbed (MpPAp model). Nevertheless, that did not led to an increased chance of reaching zero interaction probability, since the phenological coupling driver never leads to forbidden links, since all hummingbirds occurred in all months (Table 3).

For other species, as for the hummingbirds *Thalurania glaucopis* and *Aphantochroa cirrochloris*, maintaining the morphological barriers or the phenological coupling drivers unperturbed did not change the tendency of interaction probability decrease and increase respectively (Fig. 3). This occurs because both species do not present morphological or phenological traits that severely restrict their chance of interacting (Table 1, Fig. S1). Moreover, these species did not show enhanced chances of having their interaction probability reduced due to the fixation of the morphological or phenological drivers when compared to the MpPpAp model (Table 3).

4.5 DISCUSSION

The importance of morphological barriers and phenological coupling to predict observed interaction frequencies is widespread in plant-hummingbird networks (Malucelli 2014, Maruyama et al. 2014, Vizentin-Bugoni et al. 2014, Vizentin-Bugoni 2017). Still, evidences show that species abundances has some contribution to the microstructure of plant-hummingbird interaction networks as well (Malucelli 2014, Vizentin-Bugoni 2017). Here, although the best model was the one including all three interaction drivers, the probabilistic model including only species abundance was the most similar to the observed interaction frequency matrix. The neutral driver importance is possibly related to the fact that most species were not strongly constrained by long corollas or small bills. Moreover, all hummingbirds were resident, excluding the

possibility of forbidden links due to temporal mismatch. This reduce the deviation from what would be expected in a neutral process of interaction establishment.

Our simulation results suggest that in pollination networks where trait-based and neutral interaction drivers are important, the presence of restrictive pollination related traits enhance the resistance of the network to changes on the distribution of interaction probabilities across pairs of species. Nevertheless, this also means that some species, with more restrictive pollination traits, will be less flexible to cope with perturbations and might have a higher chance of reducing or completely losing its interactions. Actually, it is a widespread concept in ecology that specialized species are at greater risk in perturbation scenarios, when compared to generalists (Julliard et al. 2003, Memmott et al. 2007, Stang et al. 2007, Colles et al. 2009), although this cannot be generalized to all ecological systems (Vázquez and Simberloff 2002). This concept is supported by an observed decline of specialist species following the increasing anthropic pressure on the last decades (Clavel et al. 2011). Our results are also supported by empirical studies. For example, the South African plant, *Brunsvigia litoralis* (Amaryllidaceae), is locally endangered of extinction because of a morphological specialization. This species has a long corolla and is pollinated by only one bird species (Geerts and Pauw 2012). Urbanization lead to the local extinction of its only effective pollinator (Geerts and Pauw 2012). Although other pollinators visit this species in urban environments, they do not have long bills and act only as nectar robbers. Community studies using simulations showed a similar situation, where morphological restrictions of pollinators in a community enhanced their chance of being extinct (Stang et al. 2007). Here we build on these results showing that the specialization on other traits, such as phenology, might also pose as a disadvantage for species in scenarios of perturbations. Alternatively, for the species that are not restricted

by traits, there is the possibility of opportunistic interactions (Canard et al. 2014), what would reduce the risk for these species in face of adverse perturbations (Stang et al. 2007, Kaiser-Bunbury et al. 2010, Timóteo et al. 2016).

Here we showed that species with restrictive traits would have problems to persist in perturbed communities. Nonetheless, such species might compensate this disadvantage by other means. For example, rapid adaptive changes on pollination related traits could permit some species to persist in perturbed communities (Smith et al. 1995, Bodbyl and Kelly 2011). Another possibility is that species can migrate and establish in other localities that present adequate partners (Armbruster and Baldwin 1998). Finally, plant species might have the possibility to reproduce without the strict need of pollinators, what would reduce the effect of perturbations (Bond 1994, Fenster and Martén-Rodríguez 2007, Pauw and Bond 2011, Traveset et al. 2017). Similarly, pollinators might depend on other resources that are not related to the plants they pollinate (Junker and Blüthgen 2010).

Here we have shown contrasting consequences of perturbations in a pollination network controlled by trait-based and neutral drivers. In one hand, the maintenance of trait-based drivers unperturbed will reduce the overall change on the interactions probability distribution through the network. Nevertheless, for species that show pollination restrictive traits, this means an enhanced risk of drastically reducing the opportunities of interactions.

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4.8 TABLES

Table 1. Species morphology, temporal occurrence and abundance on the observed network, which were used to calculate abundance, phenological coupling and morphological barriers probabilistic matrices.

Plants	**Corolla depth (mm)**	**Temporal occurrence (number of months)**	**Abundance (number of flowers)**
Aechmea nudicaulis	12.20	2	586
Aechme aornata	17.18	2	62
Costus spiralis	48.66	5	56
Dahlstedtia pinnata	37.99	8	137
Heliconia farinosa	33.60	3	7
Musa ornata	37.99	10	351
Nidularium innocentii	35.00	7	775
Nidularium procerum	35.00	1	30
Psychotria nuda	33.20	8	7527
Psychotria sutterella	14.55	3	942
Spirotheca rivierii	15.25	2	314
Vriesea carinata	45.60	6	742
Vriesea incurvata	37.59	8	243

Hummingbirds	**Bill size (mm)**	**Temporal occurrence (number of months)**	**Abundance (interaction frequency)**
Amazilia versicolor	18.00	12	26
Aphantochroa cirrochloris	20.00	12	23
Ramphodon naevius	35.00	12	201
Thalurania glaucopis	19.00	12	303

Table 2. Similarity of the probabilistic models matrices to the observed interaction frequency matrix. Values close to one shows high similarity while values close to zero represents low similarity.

Models	**Similarity**
null	0.27
M	0.31
P	0.31
MP	0.36
A	0.81
MA	0.84
PA	0.85
MPA	0.86

Table 3. The chance of each species of having its interaction probability reduced and the chance of this reduction leading to a zero interaction probability, in each of the perturbed probabilistic models. The "chance" is the proportion of the simulation runs that led to decrease or zero interaction probability in relation to all simulation runs. *Costus spiralis* and *Vriesea carinata* were the species that showed the highest chance of decrease and of reaching a zero interaction probability because of their restrictive corolla depths.

Species	**Chance of decreasing the interaction probability**			**Chance of reaching zero interaction probability**		
Plant species	**MpPAp**	**MPpAp**	**MpPpAp**	**MpPAp**	**MPpAp**	**MpPpAp**
Aechmea nudicaulis	0.402	0.223	0.349	0.047	0.054	0.054
Aechmea ornata	0.131	0.100	0.157	0.058	0.058	0.058
Costus spiralis	**0.107**	**0.543**	**0.157**	**0.061**	**0.487**	**0.061**
Dahlstedtia pinnata	0.182	0.298	0.324	0.056	0.114	0.056
Heliconia farinosa	0.076	0.073	0.100	0.062	0.062	0.062
Musa ornata	0.289	0.481	0.515	0.050	0.102	0.050
Nidularium innocentii	0.461	0.496	0.622	0.046	0.045	0.046
Nidularium procerum	0.104	0.079	0.112	0.060	0.060	0.060
Psychotria nuda	0.987	0.988	0.987	0.039	0.035	0.039
Psychotria suterella	0.519	0.348	0.491	0.046	0.052	0.052
Spirotheca rivieri	0.286	0.171	0.277	0.052	0.055	0.055
Vriesea carinata	**0.267**	**0.706**	**0.399**	**0.053**	**0.422**	**0.053**
Vriesea incurvata	0.240	0.376	0.411	0.054	0.108	0.054
Hummingbird species						
Amazilia versicolor	0.051	0.123	0.081	0.000	0.003	0.000
Aphantochroa cirrochloris	0.041	0.038	0.071	0.000	0.000	0.000
Ramphodon naevius	0.765	0.677	0.750	0.000	0.000	0.000
Thalurania glaucopis	0.914	0.925	0.896	0.000	0.000	0.000

4.7 FIGURES

Figure 1.Pattern of similarity decay of each probabilistic model with increasing perturbation intensity in relation to the observed interaction frequency matrix. All models depart from a similarity of 0.86 (similarity of the MPA model).

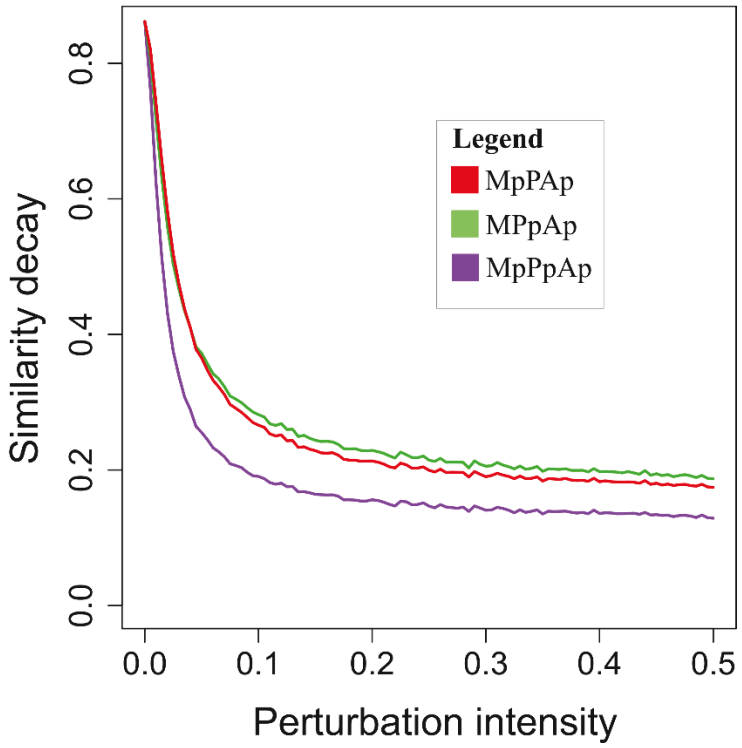


Figure 2. Convergence tendency of plant and hummingbird species mean probability of interactions when perturbing all interaction drivers (model MpPpAp) along increasing perturbation intensities. Green lines represent plant species interaction probability and blue lines represent hummingbird species interaction probability.

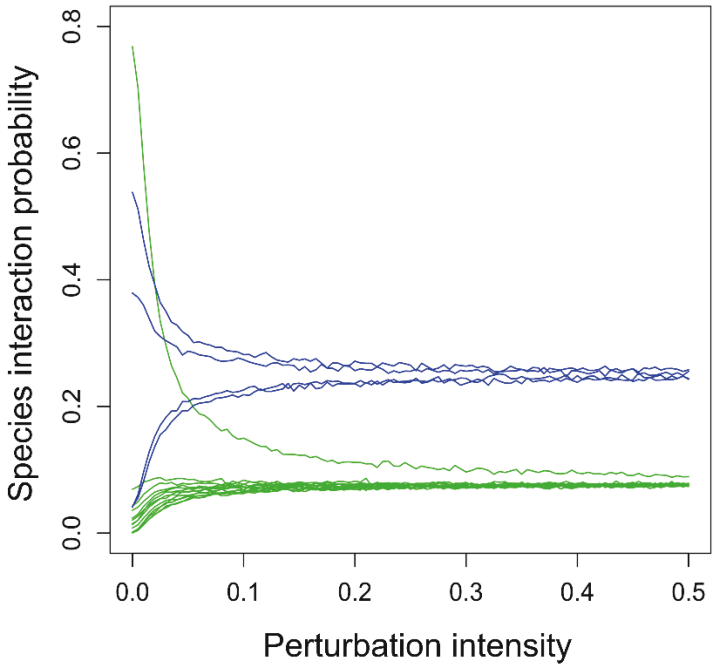
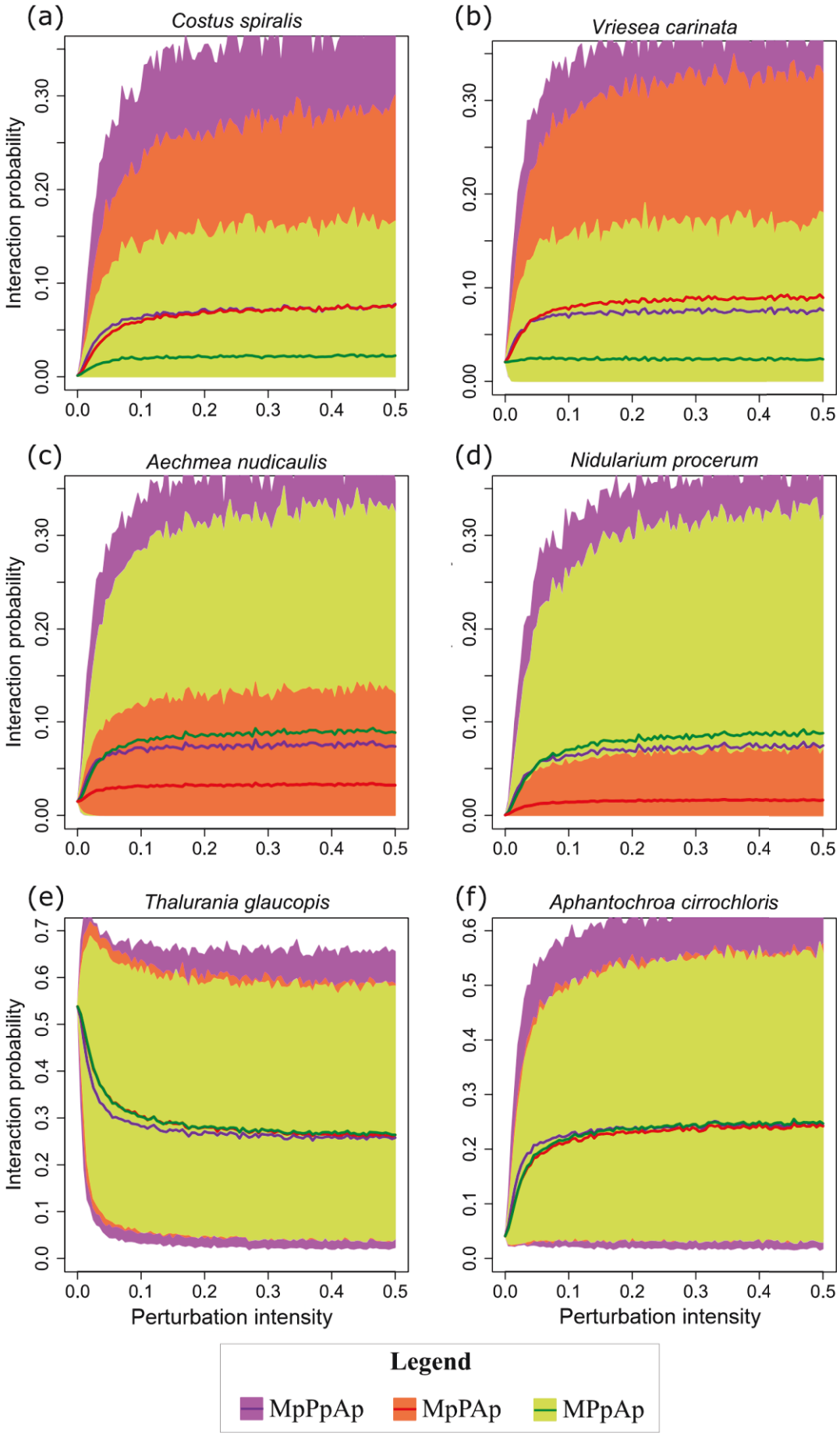


Figure 3. Mean interaction probability for the MpPpAp, MpPpAp and MpPpAp probabilistic models, for selected species along the increasing intensity perturbations. We also present for each probabilistic model upper and lower 95% confidence intervals.



4.10 SUPPLEMENTARY MATERIAL

Figure S1. Interaction probabilistic models matrices based on species abundances (a), on phenological coupling (b), on morphological barriers (c) and on the combination of the three drivers (model MPA) (d). In (e), the observed frequency of interactions. The probabilistic model including the three interaction drivers (d) was the one that best explained the observed probability of interactions. The size of the circles is proportional to the relative probability of each matrix cell.

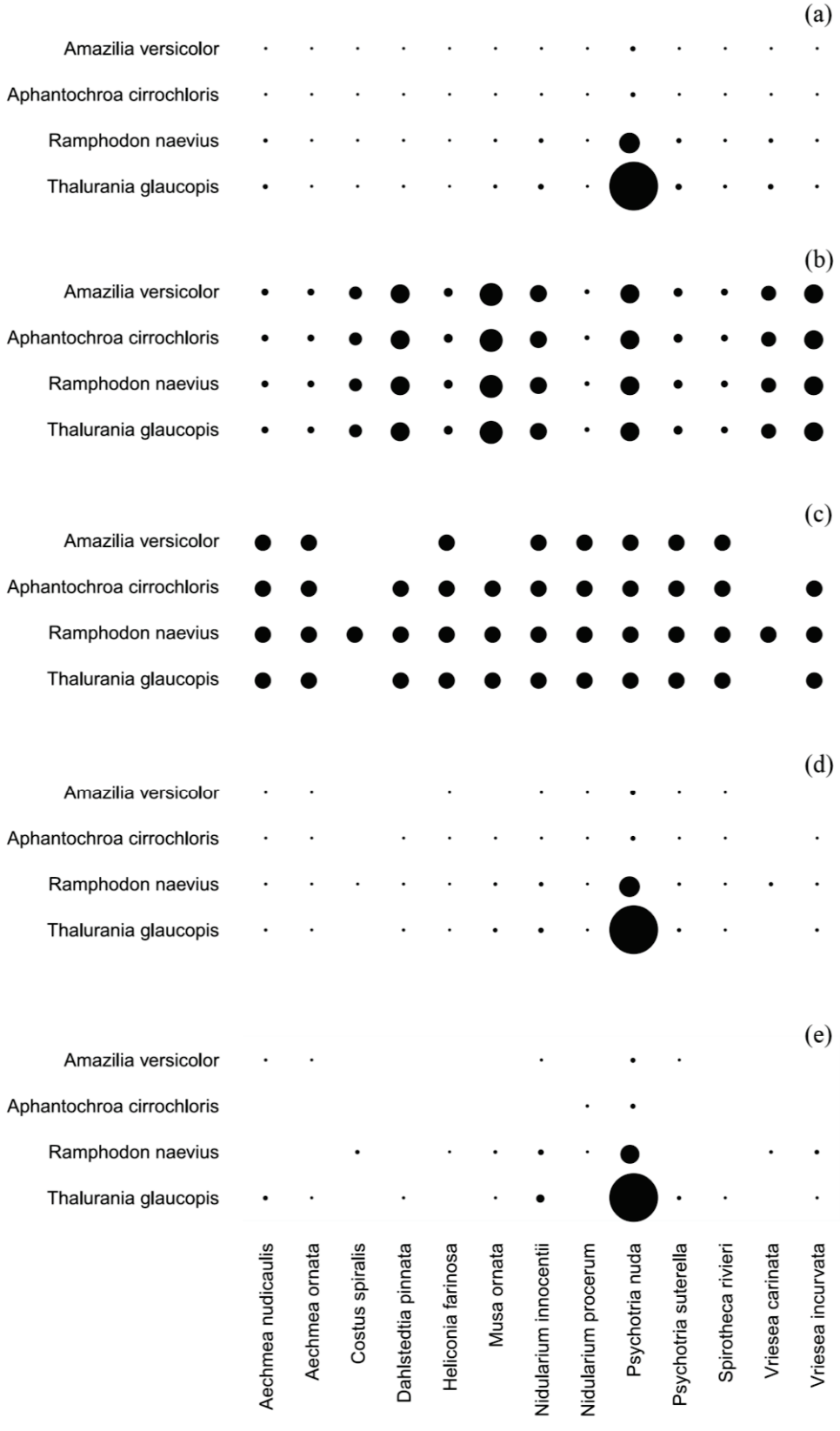


Figure S2. Mean interaction probability for the MpPpAp, MpPpAp and MpPpAp probabilistic models, for all plant species along increasing intensity perturbations. We also present for each probabilistic model upper and lower 95% confidence intervals.

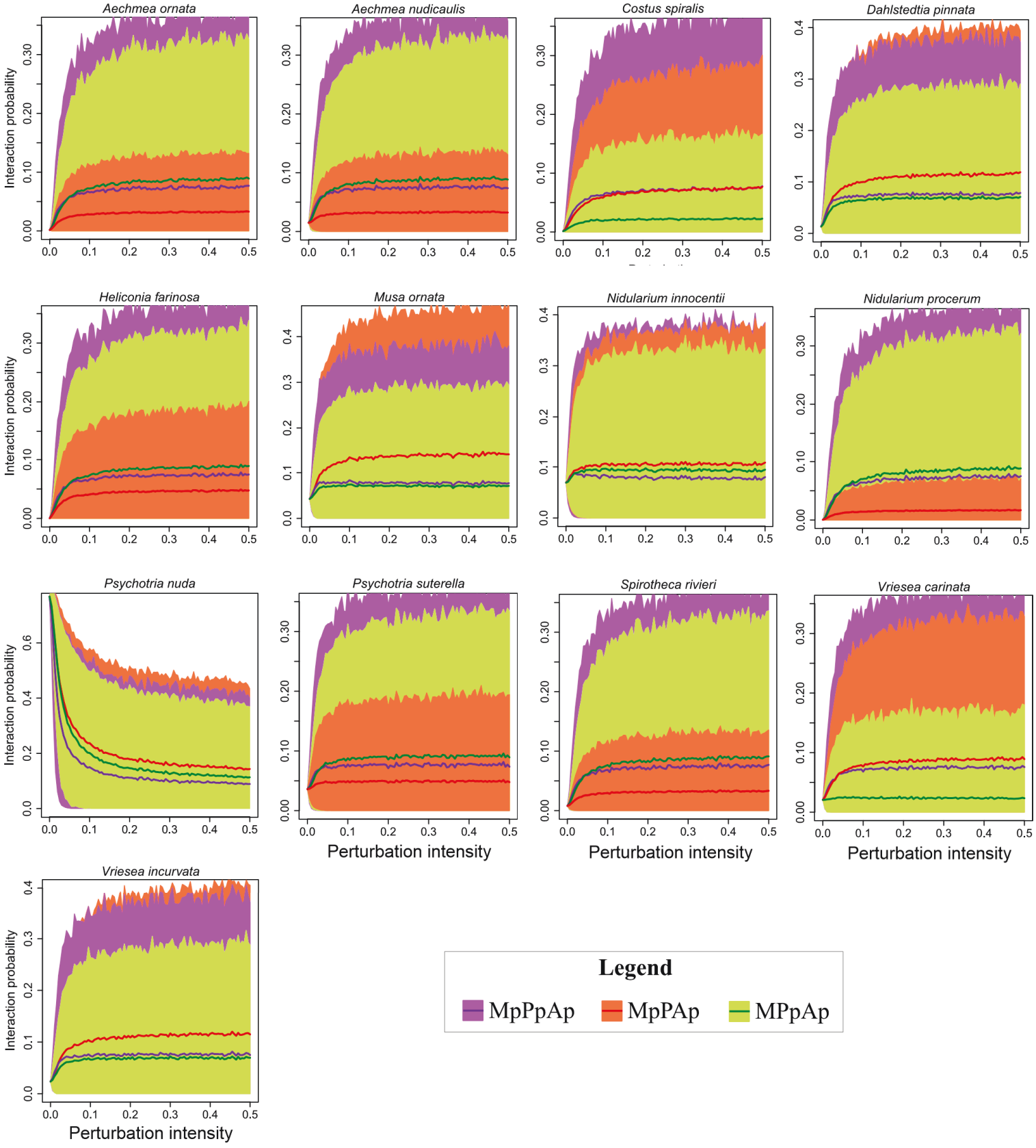
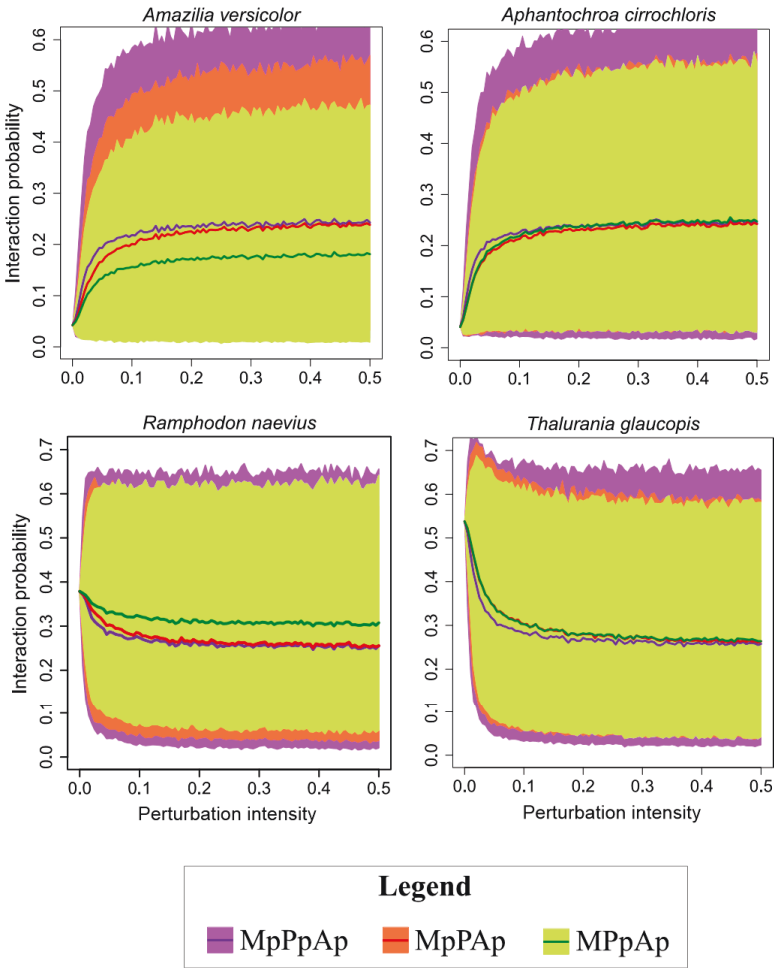


Figure S3. Mean interaction probability pattern for the MpPpAp, MpPpAp and MpPpAp probabilistic models, for all hummingbird species along the increasing intensity perturbations. We also present for each probabilistic model upper and lower 95% confidence intervals.



5 CONCLUSÃO GERAL

No Capítulo 1, a partir da análise de um grande número de redes de polinização na região Neotropical, pudemos demonstrar, empiricamente, um padrão macro ecológico de *turnover* de interações de polinização. Os resultados mostraram que em grandes escalas espaciais, o *turnover* de interações é determinado majoritariamente pelo *turnover* de espécies. Em todas as regiões biogeográficas analisadas, os gradientes ecológicos (altitude, temperatura, precipitação e distância geográfica) mostraram uma forte relação com o *turnover* de interações, sendo isso mediado por um *turnover* de plantas e beija-flores, ao invés de uma reconexão de interações entre espécies. A forte relação entre esses gradientes ecológicos e a beta diversidade de interações mediada pelo *turnover* de espécies, evidencia possíveis fatores histórico e contemporâneos na distribuição das interações plantas-beija-flor.

No Capítulo 2, observamos a influência do esforço amostral no espaço e no tempo para a detecção de interações em uma rede local. Assim como já visto em outros estudos, o esforço temporal mostrou-se bastante importante. No entanto, evidenciamos que o esforço espacial é relevante para detectar espécies menos comuns, e consequentemente suas interações. Dessa forma, o planejamento do esforço amostral no espaço deve ser cuidadosamente considerado, principalmente e locais que apresentem uma grande proporção de plantas ou polinizadores raros. Além disso, nossos resultados revelaram que a especialização de rede mensurada pelo H2’ é mais robusta do que a conectância, reforçando a ideia de que métricas quantitativas são mais seguras em casos de baixo esforço amostral. A maior importância do esforço amostral temporal mostrou que para a rede estudada, a observação de um grande número de parcelas se torna redundante. Assim, parte dos recursos investidos no esforço amostral espacial poderiam ser revertidos em um aumento do esforço temporal ou em outras atividades de pesquisa. Assim sendo, nosso estudo mostra a importância de se avaliar cuidadosamente o design amostral, para permitir um uso eficiente dos escassos recursos destinados à pesquisa.

No Capítulo três observamos os efeitos de perturbações nos determinantes de interação. Detectamos que a manutenção dos determinantes de interação relacionados aos atributos das espécies tem o potencial de diminuir a variação da distribuição das

probabilidades de interação na rede como um todo. No entanto, para espécies que possuem atributos morfológicos e fenológicos restritivos, existe um maior rico de drástica redução nas oportunidades de interação. Isso pode impedir um rápido processo de adaptação em situações de perturbação adversa. Esses resultados dão suporte à hipótese de que espécies mais especializadas sofrem um maior risco de declínio em cenários de perturbação.

Os resultados dessa tese mostraram que a ocorrência de interações entre plantas e polinizadores no espaço e no tempo podem ser explicadas por diferentes fatores que interagem de formas bastante complexas. Dessa forma, acreditamos que nossas conclusões contribuíram para avançar o estudo das interações entre espécies, particularmente entre plantas e polinizadores. No entanto, ficou claro que essa área da ecologia necessita de um grande esforço de pesquisa. Se por um lado pudemos mostrar que a variação espacial das interações está muitas vezes associada com um possível efeito de gradientes ecológicos na distribuição de plantas e polinizadores, por outro, pudemos perceber a grande influência de questões amostrais na detecção de interações. Assim, as conclusões em estudos de interações entre espécies devem ser tomadas com cautela. Mais pesquisas são necessárias para se entender melhor os efeitos amostrais em dados de interações entre espécies. Além disso, ao mostrarmos que algumas espécies podem estar mais sujeitas ao risco de extinção devido a relação de seus atributos com as interações que as mesmas fazem, revelamos a importância de se considerar, não apenas interações entre as espécies, como os atributos envolvidos nessas interações para fins de conservação e para a previsão das consequências de mudanças globais na distribuição das espécies.

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